Removal of pharmaceuticals from hospital wastewater by staged biofilm and ozone polishing

Tang, Kai

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Removal of pharmaceuticals from hospital wastewater by staged biofilm and ozone polishing

Kai Tang

PhD Thesis
December 2017

DTU Environment
Department of Environmental Engineering
Technical University of Denmark

Removal of pharmaceuticals from hospital wastewater by staged biofilm and ozone polishing

Kai Tang

PhD Thesis, December 2017

Address: DTU Environment
Department of Environmental Engineering
Technical University of Denmark
Miljoevej, Building 113
2800 Kgs. Lyngby
Denmark

Phone reception: +45 4525 1600
Fax: +45 4593 2850

Homepage: http://www.env.dtu.dk
E-mail: reception@env.dtu.dk

Cover: GraphicCo

Preface

This PhD thesis is based on research carried out in the Department of Environmental Engineering at the Technical University of Denmark from January 2015 to December 2017. It was prepared as part of the Mermiss project and performed under the main supervision of Professor Henrik Rasmus Andersen (DTU Environment) and the co-supervision of Professor Kai Bester (Aarhus University, Environmental Science).

The thesis is organised in two parts: the first part puts into context the findings of the PhD in an introductive review, while the second consists of the papers listed below. These will be referred to in the text by their paper numbers, written with the Roman numerals **I-V**.

I Ooi, G.T.H., **Tang, K.,** Chhetri, R.K., Kaarsholm, K.M.S., Sundmark, K., Kragelund, C., Litty, K., Christensen, A., Lindholst, S., Sund, C., Christensson, M., Bester, K., Andersen, H.R., 2017. Biological treatment of hospital wastewater in a pilot-scale staged Moving Bed Biofilm Reactors (MBBRs) utilizing both nitrifying and denitrifying processes. *Manuscript to be submitted*.

II **Tang, K.**, Escola Casas, M., Ooi, G.T.H., Kaarsholm, K.M.S., Bester, K., Andersen, H.R., 2017. Influence of humic acid addition on the degradation of pharmaceuticals by biofilms in effluent wastewater. *International Journal of Hygiene and Environmental Health*, **220**, 604-610.

III **Tang, K.**, Ooi, G.T.H., Litty, K., Sundmark, K., Kaarsholm, K.M.S., Sund, C., Kragelund, C., Christensson, M., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals in conventionally treated wastewater by a polishing moving bed biofilm reactor (MBBR) with intermittent feeding. *Bioresource Technology*, **236**, 77-86.

IV **Tang, K.**, Spiliotopoulou, A., Chhetri, R.K., Ooi, G.T.H., Kaarsholm, K.M.S., Sundmark, K., Florian, B., Kragelund, C., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals,

toxicity and natural fluorescence by ozonation of biological treated hospital wastewater with further polishing by suspended biofilm. *Manuscript to be submitted*.

V **Tang, K.**, Ooi, G.T.H., Chhetri, R.K., Spiliotopoulou, A., Kaarsholm, K.M.S., Sundmark, K., Florian, B., Kragelund, C., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals, toxicity and natural fluorescence by ozonation in biological pre-treated municipal wastewater in comparison to subsequent polishing biofilm reactors. *Manuscript to be submitted*.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study:

- Ooi, G.T.H., **Tang, K.**, Bester, K., Andersen, H.R., 2017. Biological treatment of municipal wastewater in a pilot-scale staged Moving Bed Biofilm Reactors (MBBRs) and MBBRs combined with activated sludge (Hybas). *Manuscript*.
- **Tang, K.**, Kragelund, C., Andersen, H.R. (2017). Removal of pharmaceuticals in conventionally treated wastewater by a polishing sand filtration with intermittent feeding. *In preparation*.
- Droumpali, A., **Tang, K.**, Litty, K., Mikkelsen, N., Lindholst, S., Kragelund, C., Andersen, H.R. (2017). Irrigation of treated wastewater in Samsø, Denmark. *In preparation*.

Furthermore, this PhD study also contributed to several international conferences with the following conferences papers:

- **Tang, K.**, Escola Casas, M., Bester, K., Andersen, H.R., Influence of dissolved organic carbon on biodegradation of pharmaceuticals by suspended biofilms in wastewater. 2nd Young Water Professionals Denmark Conference and Workshop. Aarhus (Denmark), March 10-11, 2016. Oral presentation.
- **Tang, K.**, Bester, K., Andersen, H.R., Polishing of pharmaceuticals in conventionally treated wastewater with intermittently fed Moving Bed Biofilm Reactors (MBBR). 8th INTERNATIONAL WATER & HEALTH SEMINAR. Cannes (France), June 27-29, 2016. Oral presentation.
- **Tang, K**., Ooi, G.T.H., Spiliotopoulou, A., Chhetri, R.K., Kaarsholm, K.M.S., Florian, B., Kragelund, C., Bester, K., Andersen, H.R., Pharmaceuticals, toxicity and natural fluorescence intensity of

biologically treated hospital wastewater removed by pilot and laboratory scale ozonation. 15th International Conference on Environmental Science and Technology. Rhodes (Greece), August 31 to September 2, 2017. Oral Presentation.

- **Tang, K.**, Ooi, G.T.H., Litty, K., Sundmark, K., Sund, C., Kragelund, C., Christensson, M., Bester, K., Andersen, H.R., Removing residual pharmaceuticals from activated sludge effluent by intermittently fed Moving Bed Biofilm Reactors (MBBR). 10th Micropol & Ecohazard Conference. Vienna (Austria), September 18-20, 2017. Oral presentation.
- **Tang, K.**, Ooi, G.T.H., Florian, B., Sundmark, K., Sund, C., Kragelund, C., Bester, K., Andersen, H.R., Pilot and laboratory scale ozonation of biologically treated hospital wastewater for removal of pharmaceuticals and toxicity concurrently with natural fluorescence intensity. 10th Micropol & Ecohazard Conference. Vienna (Austria), September 18-20, 2017. Oral presentation.

Acknowledgements

First, I would like to express my deep thanks to my supervisor Professor Henrik Rasmus Andersen who gave me an opportunity to be in Denmark and work on the Mermiss project. I feel so grateful always receive his nice feedbacks and advices. He is good at perceiving my needs not only for now but also for future. I am thankful to my co-supervisor Professor Kai Bester for support and guidance during my PhD. I also appreciate that people involved in the Mermiss project specially Caroline, Sabine, Kim and Alice to make the project work very well.

Then, the big thanks go to both Dr. Kamilla M.S. Kaarsholm and Dr. Ravi K Chhetri, without their warm-hearted and selfless help, I could not cope with many things alone. I am very grateful to Dongah, Aikaterini, Ariadni, Argryo, Gordon, Yuhoon and Paul, for keeping nice work atmosphere and making our group so special. A particular thank goes to Anne Harsting for supporting constantly during my PhD period. I also would like to say thanks to Mikael Emil Olsson, Sinh Hy Nguyen, Hanne Bøggild, Charlotte Lind and Kim Ryberg for their helps.

I would like to say thanks to Biao, Su, Ma, Sheng, Liguang, Liguan, Frank, Xiaohu, Xinyu, Nannan, Weichu, Peter, Sarah, David, Junxi, Tenpeng for having good time together in Denmark.

Finally, I want to thank the constant supports and loves from my family: my parents Jianzhang and Hongwei, my grandparents, my uncles and aunts, and my cousins.

Summary

Research on the removal of micropollutants (i.e. pharmaceuticals) has received a lot of attention in the last few decades. Wastewater treatment plants (WWTPs) act as the final checkpoint in controlling the quality of wastewater before discharge into receiving water. However, conventional WWTPs are not able to remove every pharmaceutical, including the majority of hardly biodegradable compounds and effluents that eventually affect the aquatic environment. Therefore, either upgrading traditional processes in WWTPs or the onsite treatment of pharmaceuticals at the point source (i.e. hospital wastewater), before release into the sewer system, must be considered, in order to overcome the above issues.

Moving bed biofilm reactors (MBBRs) as an alternative to activated sludge have been already proven highly capable of removal of pharmaceutical. Based on 36 pharmaceuticals in hospital wastewater, the concentrations of which are limited through DHI (Institute for Water and Environment), these targeted compounds are investigated in this research despite no current regulations for the presence of pharmaceuticals WWTP effluent.

Concentrations of substrate in wastewater can affect the degradation of organic micropollutants, due to a number of involved biodegradation mechanisms, including co-degradation and competitive inhibition. The effect of humic acid, as a model complex organic substrate, was investigated in relation to the biodegradation of pharmaceuticals in WWTP effluent via a laboratory-scale polishing MBBRs. Twelve investigated pharmaceuticals were significantly biodegradable. The biodegradation rate constants of ten of these compounds increased in line with increased humic acid concentrations, which shows that the presence of complex substrates stimulates degradation via a co-metabolism-like mechanism rather than competitive inhibition.

Staged MBBRs were applied for polishing of the effluent of an activated sludge treatment plant, in order to enhance the removal of pharmaceuticals. To address the issue regarding effluent not containing sufficient organic matter to sustain enough biomass, a novel feeding approach, namely intermittent feeding to MBBRs reactor with WWTP effluent and settled raw wastewater, was implemented for the first time. First-order rate constants for pharmaceutical removal, normalized to biomass, were significantly higher compared to other studies on activated sludge and suspended biofilms, especially for diclofenac, metoprolol and atenolol. Due to intermittent

feeding, diclofenac degradation occurred with a half-life of only 2.1 hours and was thus much faster than any hitherto described wastewater bioreactor treatments.

An onsite pilot-scale of staged MBBRs, involving only the MBBR technique, was applied to remove pharmaceuticals existing in raw hospital wastewater, in order to achieve relevant Danish regulation standards on discharge. Furthermore, a pilot-scale of staged MBBRs, involving MBBR and MBBR combined with activated sludge (Hybas) techniques, was applied to treat raw municipal wastewater, with the aim of attaining a high degree of pharmaceutical degradation. The strategy of intermittent feeding was carried out for both studies. In general, the majority of pharmaceuticals were removed sufficiently compared to other biological treatment processes, and the removal of diclofenac occurred in the reactors following the implementation of intermittent feeding.

A pilot ozonation system was introduced to treat effluents from the staged MBBRs that were applied to treat hospital/municipal wastewater. This was able to attain further removal of remaining pharmaceuticals and toxicity. Concentrations of pharmaceuticals decreased when ozone dosage increased, and then the ozone dose reaching 90% removal of pharmaceutical was normalized by DOC, following that relevant removal efficiency was comparable to literature studies of ozonation. Natural fluorescence as an easily measurable parameter for the oxidation of organic matter in wastewater appeared to degrade quickly along with an increase in ozone doses. Microtoxicity in the wastewater of staged MBBRs decreased along the treatment train, and ozone was able to remove half of the remaining toxicity in MBBR effluents. Polishing MBBRs applied after ozone, with the ultimate aim of reducing ozone by-products, removed almost all water toxicity.

Dansk sammenfatning

Forskning i fjernelse af mikroforureningsstoffer (f.eks. lægemidler) har fået stor opmærksomhed de seneste årtier. Spildevandsrensningsanlæg fungerer som den sidste barrier til at sikre kvaliteten af spildevand, før det udledes til recipienter. Konventionelle rensningsanlæg kan imidlertid ikke fjerne alle lægemidler, hvorved størstedelen af ikke-biologisk-nedbrydelige forbindelser udledes sammen med det behandlede spildevand, og de kan derved i sidste ende påvirker vandmiljøet. Derfor bør der enten ske en opgradering af traditionelle processer i rensningsanlæg eller en behandling af lægemidler ved kilden (dvs. behandling af hospitalsspildevand) inden udledning til kloaksystemet.

Moving Bed Biofilm Reactor (MBBR) som et alternativ til aktivt slam, har allerede vist sig at være i stand til at fjerne lægemidler. For 36 lægemidler er grænseværdier blevet foreslået i DHI (Institut for Vand og Miljø) til hospitalsaffald, på trods af manglende regler for tilstedeværelse af stoffer i kommunalt spildevand.

Koncentrationer af co-substrat i spildevand kan påvirke nedbrydningen af organiske mikroforureningsstoffer på grund af forskellige bionedbrydningsmekanismer, herunder co-nedbrydning og konkurrencebetinget inhibering. Effekten af humussyre som modelstof for et komplekst organisk substrat, blev undersøgt i relation til biologisk nedbrydning af lægemidler i renset spildevand via en laboratorieskala MBBR biofilm. Tolv undersøgte lægemidler var signifikant bionedbrydelige. De biologiske nedbrydningshastighedskonstanter for ti af disse lægemidler steg med øged koncentration af humussyre, hvilket viser at tilstedeværelsen af komplekse substrater stimulerer nedbrydning via en co-metabolisme-lignende mekanisme snarere end konkurrencepræget inhibering.

Trindelt MBBR blev anvendt til polering af spildevandet fra et aktivt slambehandlingsanlæg for at forbedre fjernelsen af lægemidler. For at løse problemet med at rense spildevand som ikke indeholder tilstrækkeligt organisk materiale til at opretholde tilfredsstillende biomasse, blev en ny fodringsmetode, nemlig intermitterende tilførsel af behandlet spildevand blandet med råt spildevand, implementeret for første gang. I biofilm dyrket på denne måde var førsteordenshastighedskonstanter for fjernelse af lægemidler, normaliseret til biomasse, signifikant højere sammenlignet med andre undersøgelser af aktivt slam og suspenderede biofilm, især for diclofenac, metoprolol og atenolol.

På grund af intermitterende fodring forekom diclofenac nedbrydning med en halveringstid på kun 2,1 timer hvilket var meget hurtigere end nogen tidligere beskrevne bioreaktorbehandling af spildevand.

Et trindelt MBBR pilotskalaanlæg blev anvendt til at fjerne lægemidler i hospitalsspildevand, for at opnå relevante danske reguleringsstandarder for udledning. Endvidere blev der anvendt et pilotanlæg med trindelt MBBR, der involverer MBBR og MBBR kombineret med aktivt slam (Hybas) til behandling af råt kommunalt spildevand med det formål at opnå en høj grad af nedbrydning af lægemidler. Strategien med intermitterende fodring blev udført for begge undersøgelser. Generelt blev størstedelen af lægemidlerne fjernet bedre sammenlignet med andre biologiske behandlingsprocesser, og fjernelsen af diclofenac forekom i reaktorerne efter implementeringen af intermitterende fodring.

Et pilot-ozoneringssystem blev opført til behandling af spildevand fra de trindelte MBBR'er, der blev anvendt til behandling af hospital/kommunalt spildevand. Dette var i stand til at opnå yderligere fjernelse af resterende lægemidler og toksicitet. Koncentrationerne af lægemidler faldt med øget ozondoseringen. Den fundne DOC normaliseret ozondose, der er nødvendig for 90% fjernelse af lægemiddel, var sammenlignelig med litteraturværdier for ozonering af behandlet spildevand. Naturlig fluorescens som en let målbar parameter for oxidation af organisk stof i spildevand, viste sig at nedbrydes hurtigt sammen med en stigning i ozon doser. Mikrotok® aktivitet i spildevandet i den trindelte MBBR-anlæg faldt i takt med behandlingen, og ozon var i stand til at fjerne halvdelen af den resterende toksicitet i MBBR-spildevandet. Polerende MBBR, som blev anvendt efter ozonbehandling med det formål at reducere ozonbiprodukter, fjernede næsten al toksiciteten.

Table of contents

Abbreviations

MBBR	Moving bed biofilm reactor
WWTP	Wastewater treatment plant
HA	Humic acid
BOD	Biochemical oxygen demand
DNAS	Denitrifying activated sludge
NMBBR	Nitrifying MBBR

1 Introduction

1.1 Background and approach

Due to the high consumption of pharmaceuticals in the last few decades, their widespread presence in wastewater has attracted a great deal of attention (Herrmann et al., 2015). However, conventional wastewater treatment plants (WWTPs) as the last obstacle were not able to completely degrade all pharmaceuticals before discharging into receiving water sources (Verlicchi et al., 2012b). Thus, contaminated WWTP effluent can affect the aquatic environment (Overturf et al., 2015). Consequently, moving bed biofilm reactors (MBBRs), as a recently biological technology, can be considered to address this issue (Ødegaard, 2006).

MBBRs consist of flow-through wastewater and suspended plastic carriers, on which attached biofilms can grow. Previous studies have proven that MBBRs remove more pharmaceuticals than activated sludge (Escolà Casas et al., 2015a; Falås et al., 2012). To upgrade conventional WWTPs and enhance the chances of pharmaceutical removal, MBBRs can be used in two ways as an alternative to activated sludge. On the one hand, in order to partly or fully replace traditional wastewater treatment processes, MBBRs applied as onsite treatment solutions for hospital wastewater are able to ease the processing load for pharmaceutical removal for WWTPs, or fully municipal wastewater is received by MBBRs instead of activated sludge. On the other hand, MBBRs can be applied to polish WWTP effluent and thus remaining pharmaceuticals present in effluent wastewater are able to be further removed before discharge.

For the first option, although MBBRs perform well in removing pharmaceuticals, the polishing process is still demanded after MBBRs, and thus hardly biodegradable pharmaceuticals can be removed from MBBR effluent. Ozone, with matured experiences of operation and efficient cost, is considered as a feasible solution to polish effluent wastewater (Hansen et al., 2016; Hollender et al., 2009). However, ozone by-products with even higher toxicity compared to original compounds cannot be avoided during ozone reaction. Besides examining removal of pharmaceutical via treatment processes, toxicity in wastewater also needs be investigated, as it would clearly reveal the entirely hazardous nature of wastewater and evaluate thoroughly the performance of treatment processes. Furthermore, fluorescence technique, with advantages of rapid analysis and non-need for reagents, has been provided a

tool to monitoring of DOC fraction in wastewater and determine indirectly ozone in recirculating aquaculture systems water (Hudson et al., 2008; Spiliotopoulou et al., 2017). While, the knowledge gap, which addresses the correlation between fluorescence intensity and ozone dose in wastewater, need be understood.

For the second aspect, WWTP effluent normally has a small amount of available carbon, and both biofilm growth and bacteria community certainly are related to the concentration and type of available carbon. As such, there is currently a lack of knowledge on how the available carbon affects the biodegradation of pharmaceuticals by polishing MBBRs. Additionally, in the staged MBBRs, less activity in relation to pharmaceutical removal occurs in the last stage, due to the lack of sufficient carbon source to support biofilm growth. Therefore, to enhance the capacity of pharmaceutical removal in the last stage, along with improving the entire removal process in staged MBBRs, issues regarding the lack of sufficient biomass need be solved.

1.2 Research aims

This research is part of the Mermiss project that focuses mainly on addressing the issue of pharmaceutical removal in different types of wastewater, including effluent in conventional WWTPs, raw hospital wastewater and municipal wastewater. Degraders responsible for removing pharmaceuticals in wastewater have been identified as less active in the last stages of MBBRs and rely positively on available carbon source from the wastewater to support their growth. However, effluents or the last stage of MBBRs normally contain less carbon compared to raw wastewater and cannot provide sufficient carbon to promote degrader growth. To have more efficient degraders in MBBRs, and eventually to obtain a high degree of pharmaceutical removal, solutions need to be found to solve issues about the lack of carbon. Moreover, optimised ozone dosages, applied in MBBR effluents to enhance the removal of non-biologically degradable compounds, need be understood as well. What's more, the removal efficiency of compounds in MBBRs followed by ozone is not the only goal or index that needs attention, as toxicity development in ozonated wastewater should also be examined. A feasible and efficient process that is able to reduce the relevant toxicity of ozonated wastewater before discharge ought to be considered.

An overview of the approach used in this research is presented in Figure 1.1.

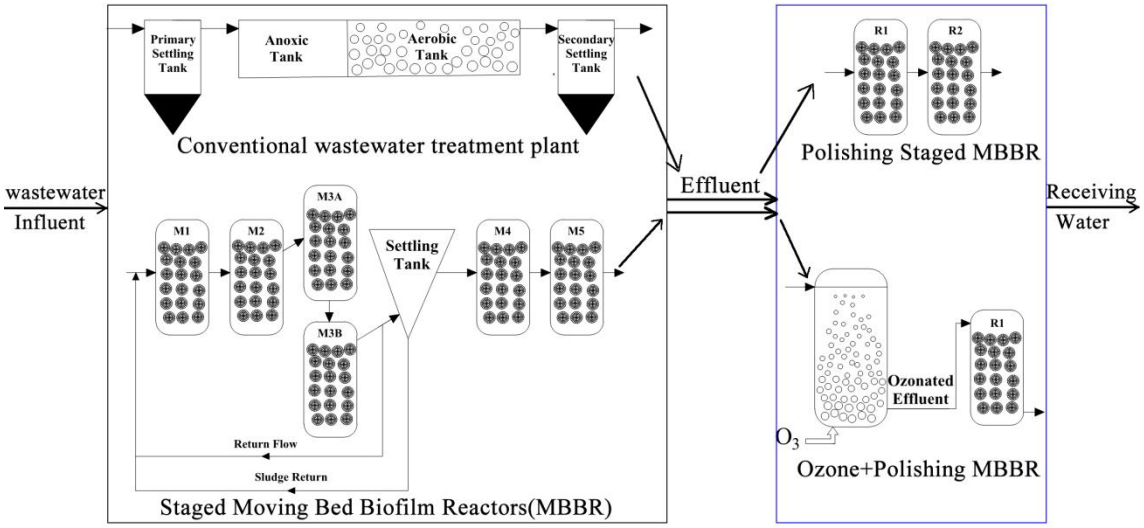

Figure 1.1. Overview of the research approach in this thesis.

The detailed aims of this research are as follows:

- Investigate the removal efficiency of pharmaceuticals in an onsite pilot of staged MBBRs, which was applied for treating hospital wastewater. Evaluate whether intermittent feeding to M3A/M3B with M2 effluent can enhance the removal of pharmaceuticals in M3. (**Paper I**)
- Investigate the effect of an additional carbon source on the removal of pharmaceutical in effluent wastewater via a laboratory-scale MBBR. (**Paper II**)
- Evaluate whether intermittent feeding to a laboratory-scale polishing MBBR with raw settled wastewater and effluent wastewater will enhance the removal of pharmaceuticals in effluent wastewater. (**Paper III**)
- Assess the effect of ozone dosage on the removal of pharmaceutical in MBBR effluent via pilot-scale and laboratory-scale ozone. The fluorescence intensity of ozonated wastewater for both the pilot and the laboratory experiments was measured under specific wavelengths, and the toxicity of wastewater in the pilot treatment process, and ozonated wastewater, was investigated. (**Paper IV**)
- Assess the effect of ozone dosage on the removal of pharmaceutical in MBBR effluent via pilot-scale and laboratory-scale ozone. The fluorescence intensity of ozonated wastewater for both the pilot and the laboratory tests was measured under specific wavelengths, and the toxicity of wastewater in the pilot treatment process, and ozonated wastewater, was investigated. Also established whether a pilot-scale polishing MBBR ap-

plied after ozonation will affect toxicity and fluorescence intensity. (**Paper V**)

2 Pharmaceuticals in hospital/municipal wastewater, removed by a pilot-scale staged Moving Bed Biofilm Reactors (MBBRs)

2.1 MBBRs system and experimental procedures

A six staged pilot-scale MBBRs, receiving raw wastewater from University Hospital (Skejby, Denmark), was carried out to test the efficiency of removing pharmaceuticals. This MBBRs system includes six reactors in total under anaerobic and aerobic conditions (Figure 2.1, left). M1 with 900L and M4 with 500L acted as denitrification reactors, while M2 and M3A/B with 900L, together with M5 with 500L, and were nitrification reactors. M1 and M4 responded to the removal of nitrate, and M5 was used as a polishing process for organic matter in denitrificated M4 effluent. The main task of M2 was to remove TOC, while pharmaceutical removal normally occurred in the M3A/B reactors during the nitrification processes. Based on the good pharmaceutical removal obtained from a novel strategy involving intermittent feeding to the reactors, and that this strategy was applied for the first time in this study, as described in Chapter 3, the feed flow from the M2 effluent to the third reactor (M3) was switched twice per day, in that either the flow went M3A first and then to M3B or the opposite way within 12 hours. Thus, biomass in the third reactor (M3A/B) would be promoted due to interchange feeding. The inlet flow rate and return flow rate were 800 L/h and 500 L/h, respectively.

Additionally, the same staged pilot-scale MBBRs from above, albeit with a different treatment configuration was moved afterward to another place (Herning, Denmark) and raw municipal wastewater treated accordingly. Rather than the case where each reactor used in Skejby was a pure MBBR technique, the staged MBBRs applied at Herning consisted of a pure MBBR and a combination of MBBR and activated sludge (Hybas). Hence, this system, for denitrification, included an activated sludge reactor of 900 L (M1) and an MBBR reactor of 500 L (M4); however, for the nitrification process, it included an activated sludge tank of 900 L (M2), Hybas reactors of 900 L (M3A and M3B) and a MBBR reactor of 500 L (M5) (Figure 2.2, left). Intermittent feeding to M3A/B was also applied in this study. The inlet flow

rate, return flow rate and return sludge flow rates were 250 L/h, 500 L/h and 300 L/h, respectively.

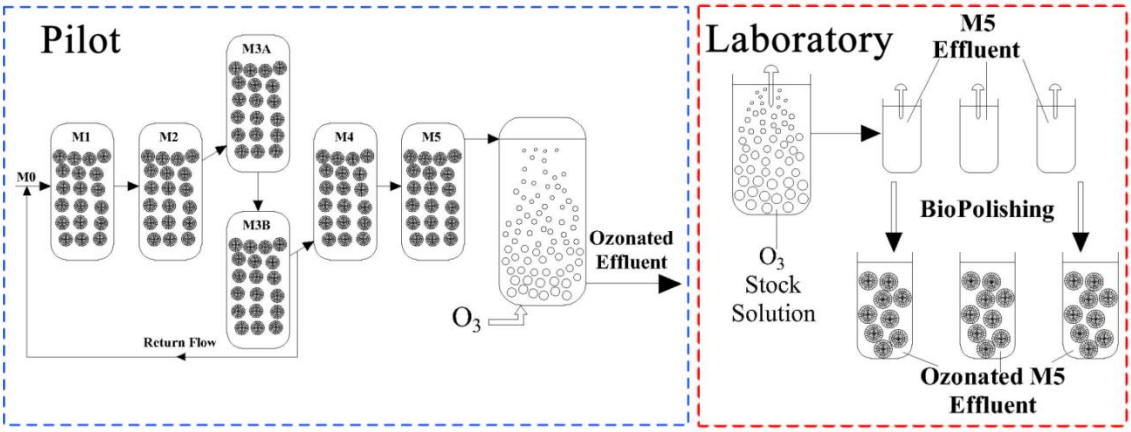

Figure 2.1. Schematic diagram of a five-staged pilot-scale MBBR treatment train followed by pilot-scale ozonation (left). M0 stands for the hospital wastewater inlet. A lab-scale MBBR was used to polish ozonated effluent in the laboratory (right). (**Paper IV**)

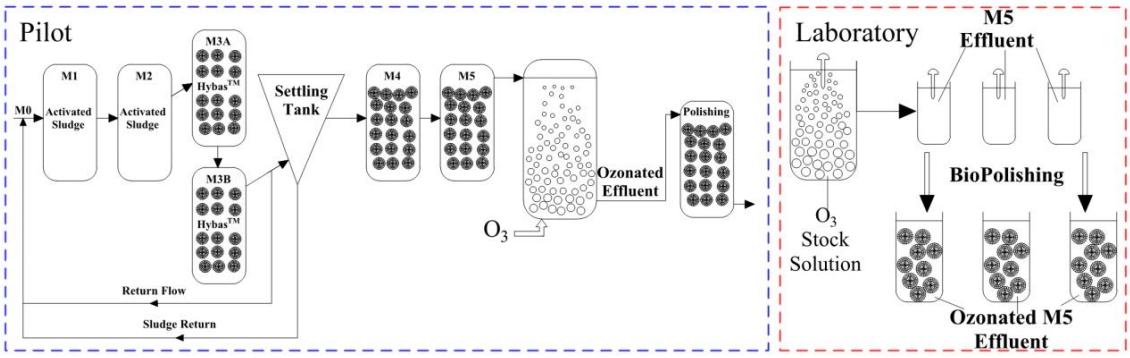

Figure 2.2. Schematic diagram of a five-stage pilot-scale MBBR treatment train followed by pilot-scale ozonation and an MBBR (left). M0 stands for the municipal wastewater inlet. A lab-scale MBBR was used to polish ozonated effluent in the laboratory (right). (**Paper V**)

To test the capability of these two MBBRs systems on pharmaceutical removal, batch experiments and continuous flow experiments were carried out. On the one hand, in the batch experiments, due to the need to spike the stock solution of pharmaceuticals in a realistic way, either carriers with the same filling ratios between the amount of carriers and the volume of wastewater or activated sludge, which depended on the individual treatment process of the reactor itself, were taken back to the laboratory, following which similar operating conditions in field were simulated by using a resemble 3 L of reactor. Then, air pumps were used to create aerobic conditions, while nitrogen gas

was pumped into the reactor to maintain these anaerobic conditions. After spiking, samples were taken over time.

On the other hand, in the continuous flow experiments, samples were taken from the influent and reactor effluent according to hydraulic retention time, and spiking would not execute because the actual behaviour of pharmaceutical removal in each reactor needed to be investigated in real-life conditions.

2.2 Potential and actual capacity of pharmaceutical removals by MBBRs

As the same experimental strategy was applied to both the hospital wastewater MBBRs (Skejby) and the municipal wastewater MBBRs (Herning), besides the differences in receiving wastewater and treatment processes applied for each reactor, the results for data treatment were similar, and therefore the following will focus only on experiments carried out with the hospital wastewater MBBRs as an exemplar. The targeted pharmaceuticals investigated in this study, as well as following studies, were classified into different groups: antibiotics (i.e. ciprofloxacin, sulfadiazine, sulfamethizole, trimethoprim, azithromycin and the sulfadiazine metabolite acetyl-sulfadiazine), blood pressure regulators (i.e. atenolol, metoprolol, propranolol and sotalol), analgesics (i.e. carbamazepine, diclofenac, ibuprofen, phenazone and tramadol), antidepressants (i.e. venlafaxine) and X-ray contrast media (i.e. iopromide, iohexol and iopamidol). The details of pharmaceuticals in stock solution and relevant suppliers are presented in the Supplementary Information of Paper II. Common parameters of wastewater in the Skejby MBBRs, along with months of operation, are illustrated in Table 2.1. The highest biomass was observed in the first denitrifying reactor (M2), due to sufficient carbon in the relevant influent, and yet the biomass in M3A and M3B was identical because of interchangeable feeding to these two reactors with M2 effluent for an equal amount of time. Almost 90% of TOC was removed by the MBBRs and a high level of NH_4^+–N and NO_2^--N was removed as well, which indicates good nitrification and denitrification achieved through MBBRs treatment.

For the batch experiments, the concentrations of analysed pharmaceuticals over time were plotted based on first-order kinetics (**2.1**), and six representative compounds are presented in Figure 2.3.

$$C = C_0 \cdot e^{-kt} \quad \textbf{(2.1)}$$

Table 2.1. Common parameters of wastewater in the Skejby staged MBBRs. (**Paper I**)

Reactor	HRT [h]	Biofilm [g/L]	pH	DO [mgO/L]	TOC [mgC/L]	NH_4^+–N [mgN/L]	NO_2^-–N [mgN/L]	NO_3^-–N [mgN/L]
Influent			7.9±0.3	2.6±1.7	137±45	49.3±14.2	0.06±0.0	0.6±0.2
M1	1.13	2.84	7.9±0.3	0.5±0.1	55±32	30.5±13.7	0.06±0.0	0.7±0.3
M2	1.13	5.13	7.8±0.2	4.9±2.4	16±3	19.5±13.1		0.4±0.0
M3A	1.13	3.23	7.7±0.3	6.8±2.4	18±6	5.4±6.6		12.3±6.8
M3B	1.13	3.23	7.6±0.3	5.7±3.1	19±10	4.8±7.2		13.7±4.2
M4	1.67	2.45	7.8±0.3	0.6±0.4	17±4	6.5±8.8	0.24±0.2	0.9±0.7
M5	1.67	3.33	8.0±0.2	7.1±2.7	16±3	4.3±7.7		2.3±0.4

In general, the higher level of pharmaceutical removal occurred in the nitrifying MBBRs (M2, M3 and M5) as opposed to the denitrifying MBBRs and the fast degradation of pharmaceuticals was observed in M3A/B. However, for trimethoprim, high removal levels were found in the denitrifying MBBRs rather than the nitrifying MBBRs. Additionally, and based on the fitting results of first-order kinetics, the degradation rate constant (k) of each pharmaceutical was obtained (Table 2.2). The highest k values were often found in M2 and M3A/B, namely, the majority of pharmaceuticals were degraded in these two nitrifying reactors.

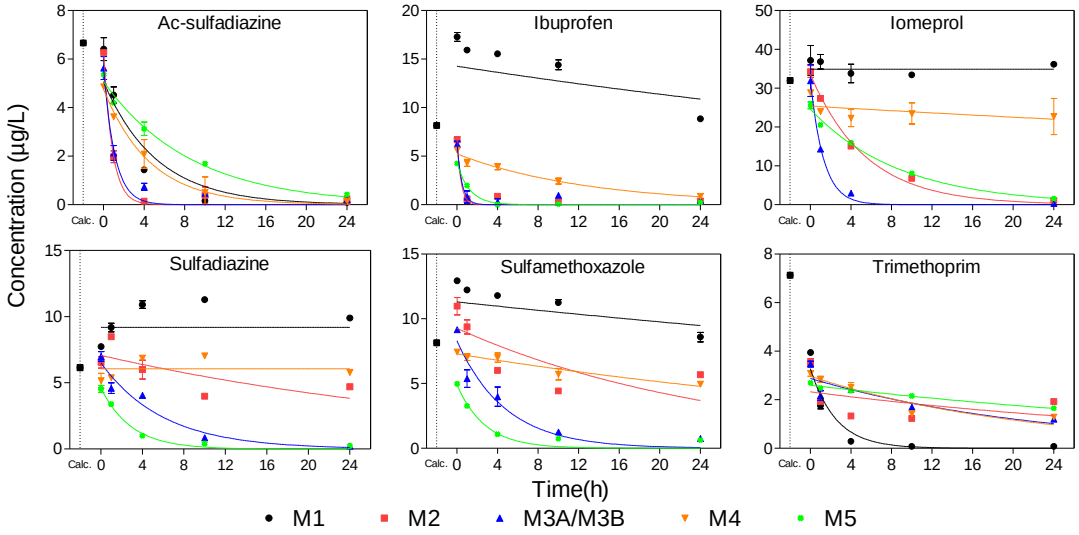

Figure 2.3. First-order reaction rate fitting to concentrations of selected pharmaceuticals in batch experiments. The dashed horizontal line stands for the limit of quantification (LOQ) as derived from the lower of two multiple reaction monitoring (MRM) signals. (**Paper I**)

For the continuous flow experiments, Figure 2.4a shows an overview of how much was removed from each reactor, while the entire MBBRs removal for from influent to effluent is illustrated in Figure 2.4b. The M5 reactor generally removed a major amount of pharmaceuticals despite low carbon levels

compared with other front reactors. It was assumed that biofilms need to utilise the hardly carbon or even the energy produced by the transformation of pharmaceuticals to support their growth. Detected compounds above LOQs normally achieved more than 50% removal. Diclofenac, known as a hardly biodegradable compound (Joss et al., 2006), achieved 80% removal. However, the average removal of diclofenac in the activated sludge and membrane bioreactor (MBR) was 36% and 48%, respectively (Vieno and Sillanpää, 2014). In Figure 2.4a, the negative removal of sulfamethizole and sulfamethoxazole happened in the first MBBR reactors (i.e. M1). This phenomenon could be explained due to conjugated compounds derived from sulfamethizole and sulfamethoxazole attaching to a sulfo group, an acetyl group or a glucuroic acid component (Berger et al., 2008). Furthermore, in the presence of relevant bacterial enzymes in wastewater, the de-conjugation process occurs, and thus conjugated compounds de-conjugate with respective groups and transform back to sulfamethizole or sulfamethoxazole.

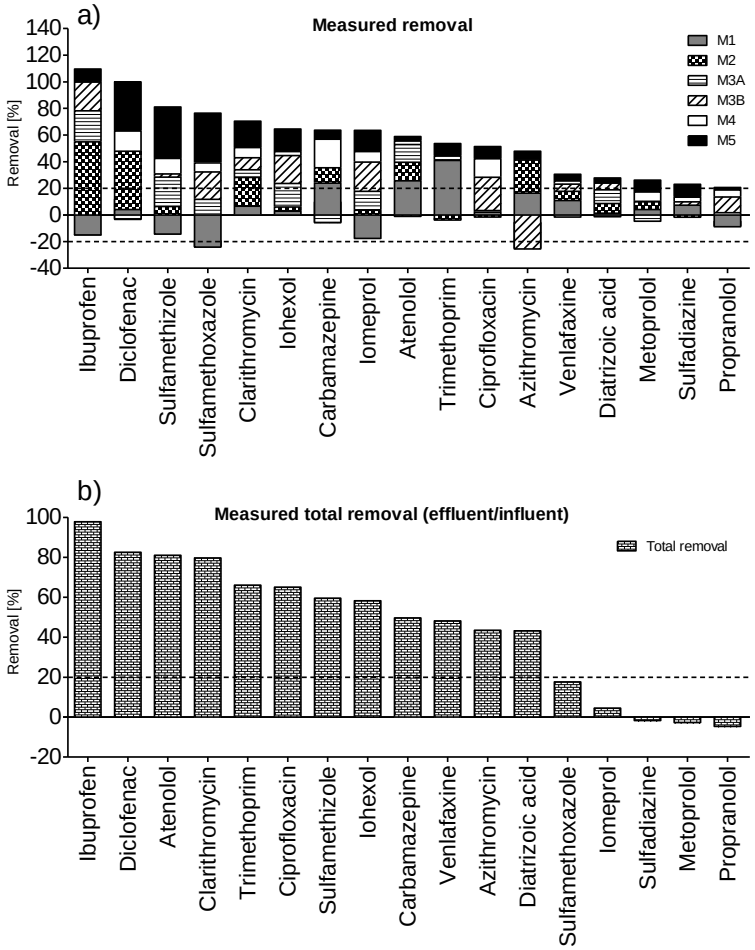

Figure 2.4. a) An overview of the removal contribution of each reactor; b) an overview of the entire removal from influent to effluent. (**Paper I**)

Table 2.2. Removal rate constant, k_i, of pharmaceuticals in each reactor. (**Modified version from Paper** I)

Compounds	M1		M2		M3A/M3B		M4		M5	
	k_{M1} [h^{-1}]	r^2	k_{M2} [h^{-1}]	r^2	k_{M3} [h^{-1}]	r^2	k_{M4} [h^{-1}]	r^2	k_{M5} [h^{-1}]	r^2
Acetyl-sulfadiazine	$19.2\pm5.2\times10^{-2}$	0.92	$\mathbf{11.7\pm1.3\times10^{-1}}$	0.99	$9.1\pm2.1\times10^{-1}$	0.97	$21.9\pm1.9\times10^{-2}$	0.99	$11.6\pm1.4\times10^{-2}$	0.99
Atenolol	$11.8\pm4.4\times10^{-3}$	0.69	$48.5\pm3.1\times10^{-2}$	1.00	$\mathbf{7.9\pm1.4\times10^{-1}}$	0.98	$23.0\pm2.3\times10^{-2}$	0.99	$28.4\pm2.0\times10^{-2}$	1.00
Azithromycin	$12.5\pm4.5\times10^{-2}$	0.85	$\mathbf{5.7\pm5.1\times10^{-1}}$	0.30	$6.0\pm4.3\times10^{-2}$	0.53	$4.8\pm1.3\times10^{-1}$	0.96	$25.1\pm2.9\times10^{-3}$	0.97
Carbamazepine	$6.3\pm7.1\times10^{-3}$	0.17	$5.5\pm17.4\times10^{-3}$	0.03	$\mathbf{1.4\pm1.5\times10^{-2}}$	0.26	$2.5\pm5.5\times10^{-3}$	0.06	$49.7\pm9.8\times10^{-4}$	0.90
Ciprofloxacin	$1.3\pm0.0\times10^{-16}$	0.00	$\mathbf{1.5\pm3.0\times10^{-2}}$	0.06	$8.1\pm15.9\times10^{-3}$	0.08	$1.7\pm0.0\times10^{-16}$	0.00	$9.6\pm7.2\times10^{-3}$	0.40
Clarithromycin	$11.0\pm4.2\times10^{-2}$	0.85	$44.6\pm5.1\times10^{-2}$	0.99	$\mathbf{5.3\pm2.2\times10^{-1}}$	0.90	$49.8\pm2.9\times10^{-2}$	1.00	$71.7\pm6.2\times10^{-3}$	0.99
Diatrizoic acid	$2.2\pm0.0\times10^{-16}$	0.00	$2.68\pm157\times10^{-4}$	0.00	$9.5\pm14.1\times10^{-3}$	0.13	$\mathbf{10.1\pm3.7\times10^{-3}}$	0.73	$2.8\pm2.8\times10^{-3}$	0.26
Diclofenac	$1.7\pm0.0\times10^{-16}$	0.00	$7.1\pm0.0\times10^{-14}$	0.00	$\mathbf{2.4\pm1.2\times10^{-2}}$	0.64	$3.4\pm8.1\times10^{-3}$	0.05	$1.5\pm0.0\times10^{-16}$	0.00
Ibuprofen	$1.1\pm1.6\times10^{-2}$	0.14	$\mathbf{2.6\pm1.1\times10^{0}}$	0.97	$21.0\pm8.2\times10^{-1}$	0.95	$7.7\pm1.3\times10^{-2}$	0.97	$81.5\pm9.2\times10^{-2}$	0.99
Iohexol	$1.3\pm0.0\times10^{-16}$	0.00	$22.5\pm1.9\times10^{-2}$	1.00	$\mathbf{111\pm8\times10^{-2}}$	1.00	$67.7\pm8.7\times10^{-4}$	0.96	$166\pm4\times10^{-3}$	1.00
Iomeprol	$1.7\pm0.0\times10^{-16}$	0.00	$18.4\pm1.6\times10^{-2}$	0.99	$\mathbf{76.8\pm8.2\times10^{-2}}$	1.00	$6.0\pm5.8\times10^{-3}$	0.27	$11.3\pm1.1\times10^{-2}$	0.99
Iopamidol	$2.3\pm2.3\times10^{-3}$	0.21	$2.04\pm168\times10^{-4}$	0.00	$\mathbf{2.0\pm1.5\times10^{-2}}$	0.43	$4.1\pm2.2\times10^{-3}$	0.54	$4.5\pm2.0\times10^{-3}$	0.64
Iopromide	$4.2\pm2.2\times10^{-1}$	0.42	$4.4\pm1.1\times10^{-1}$	0.97	$\mathbf{14.8\pm4.2\times10^{-1}}$	0.96	$9.2\pm4.5\times10^{-3}$	0.61	$19.6\pm4.5\times10^{-2}$	0.96
Metoprolol	$2.2\pm0.0\times10^{-16}$	0.00	$\mathbf{11.6\pm3.1\times10^{-2}}$	0.92	$8.9\pm3.7\times10^{-2}$	0.83	$2.7\pm3.7\times10^{-3}$	0.16	$15.1\pm2.8\times10^{-3}$	0.92
Phenazone	$1.8\pm0.0\times10^{-16}$	0.00	$\mathbf{24.9\pm6.7\times10^{-2}}$	0.94	$5.1\pm2.6\times10^{-2}$	0.67	$1.5\pm0.0\times10^{-16}$	0.00	$5.1\pm1.5\times10^{-3}$	0.79
Propranolol	$3.7\pm2.2\times10^{-2}$	0.47	$\mathbf{2.6\pm2.3\times10^{-1}}$	0.25	$3.4\pm2.9\times10^{-2}$	0.36	$3.0\pm1.6\times10^{-2}$	0.59	$1.4\pm1.3\times10^{-2}$	0.29
Sotalol	$1.6\pm2.2\times10^{-3}$	0.12	$\mathbf{6.9\pm3.0\times10^{-2}}$	0.74	$4.3\pm2.4\times10^{-2}$	0.62	$1.4\pm2.0\times10^{-3}$	0.14	$189\pm8\times10^{-4}$	1.00
Sulfadiazine	$1.7\pm0.0\times10^{-16}$	0.00	$2.6\pm1.6\times10^{-2}$	0.51	$16.6\pm4.6\times10^{-2}$	0.94	$1.9\pm0.0\times10^{-16}$	0.00	$\mathbf{34.3\pm4.5\times10^{-2}}$	0.99
Sulfamethizole	$2.9\pm2.4\times10^{-2}$	0.29	$12.6\pm4.7\times10^{-2}$	0.85	$\mathbf{9.7\pm2.6\times10^{-1}}$	0.96	$10.0\pm7.8\times10^{-3}$	0.36	$69\pm3\times10^{-2}$	1.00
Sulfamethoxazole	$7.3\pm9.5\times10^{-3}$	0.15	$3.8\pm2.3\times10^{-2}$	0.52	$21.0\pm6.5\times10^{-2}$	0.93	$17.6\pm2.8\times10^{-3}$	0.94	$\mathbf{3.6\pm1.0\times10^{-1}}$	0.94
Trimethoprim	$\mathbf{39.8\pm5.1\times10^{-2}}$	0.98	$2.3\pm3.2\times10^{-2}$	0.14	$4.4\pm1.9\times10^{-2}$	0.74	$4.7\pm1.2\times10^{-2}$	0.89	$19.2\pm2.0\times10^{-3}$	0.97
Venlafaxine	$1.7\pm0.0\times10^{-16}$	0.00	$\mathbf{3.2\pm2.6\times10^{-2}}$	0.35	$2.4\pm2.4\times10^{-2}$	0.29	$9.5\pm2.8\times10^{-3}$	0.81	$5.0\pm4.9\times10^{-3}$	0.26

2.3 Comparison of rate constants normalised by biomass

To evaluate the differences in performance relating to pharmaceutical removal, between the currently staged MBBRs and other bioreactor treatment studies, the rate constant of pharmaceuticals in each reactor was normalised to the corresponding biomass in the respective reactor, and thus k_{bio} was calculated (Table 2.3). High k_{bio} values, namely the most efficient biofilm responsible for pharmaceutical removal, were also observed in M3, which was according to the results of rate con-

stants of pharmaceuticals obtained from batch experiments and further strengthened the novel strategy that the intermittent feeding to reactors fully was applicable even at the pilot stage.

Table 2.3. Removal rate constant normalised with biomass, $k_{bio,i}$, of pharmaceuticals in each reactor. (**Modified version from Paper** I)

Compounds	$k_{bio, M1}$ [L h^{-1} g^{-1}]	$k_{bio, M2}$ [L h^{-1} g^{-1}]	$k_{bio, M3}$ [L h^{-1} g^{-1}]	$k_{bio, M4}$ [L h^{-1} g^{-1}]	$k_{bio, M5}$ [L h^{-1} g^{-1}]	Literature	Ref[1]	Conditions
Acetyl-sulfadiazine	6.76×10^{-2}	2.28×10^{-1}	**2.81×10^{-1}**	8.94×10^{-2}	3.48×10^{-2}			
						$2.79-3.75 \times 10^{-2}$	*A*	NMBBR
Azithromycin	4.40×10^{-2}	1.11×10^{-1}	1.86×10^{-2}	**1.96×10^{-1}**	7.54×10^{-3}			
						$\leq 4.17 \times 10^{-3}$	*A*	NMBBR
Ciprofloxacin	4.68×10^{-17}	2.83×10^{-3}	2.49×10^{-3}	7.10×10^{-17}	**2.88×10^{-3}**			
						$7.50-12.1 \times 10^{-3}$	*A*	NMBBR
Diatrizoic acid	7.82×10^{-17}	5.22×10^{-5}	2.95×10^{-3}	**4.12×10^{-3}**	8.38×10^{-4}			
Diclofenac	6.06×10^{-17}	1.38×10^{-14}	**7.49×10^{-3}**	1.37×10^{-3}	4.35×10^{-17}	$\leq 4.17 \times 10^{-3}$	*A*	DNAS
						$< 1.67 \times 10^{-3}$	*B*	DNAS
						4.17×10^{-3}	*C*	DNAS
Ibuprofen	3.98×10^{-3}	5.13×10^{-1}	**6.50×10^{-1}**	3.16×10^{-2}	2.45×10^{-1}	6.25×10^{-2}	*B*	DNAS
						$0.00-6.46 \times 10^{-1}$	*D*	NMBBR
Iohexol	4.72×10^{-17}	4.39×10^{-2}	**3.44×10^{-1}**	2.76×10^{-3}	4.98×10^{-2}			
Iomeprol	5.92×10^{-17}	3.59×10^{-2}	**2.38×10^{-1}**	2.44×10^{-3}	3.39×10^{-2}			
Iopamidol	8.13×10^{-4}	3.98×10^{-5}	**6.32×10^{-3}**	1.67×10^{-3}	1.34×10^{-3}			
Iopromide	1.49×10^{-1}	8.58×10^{-2}	**4.58×10^{-1}**	3.74×10^{-3}	5.89×10^{-2}			
						$2.88-3.17 \times 10^{-2}$	*A*	NMBBR
						$\leq 4.17 \times 10^{-3}$	*A*	NMBBR
Propranolol	1.30×10^{-2}	**5.01×10^{-2}**	1.04×10^{-2}	1.23×10^{-2}	4.29×10^{-3}			
Sotalol	5.60×10^{-4}	**1.35×10^{-2}**	1.33×10^{-2}	5.59×10^{-4}	5.68×10^{-3}			
Sulfadiazine	5.88×10^{-17}	4.99×10^{-3}	5.14×10^{-2}	7.92×10^{-17}	**1.03×10^{-1}**			
Sulfamethizole	1.01×10^{-2}	2.46×10^{-2}	**3.01×10^{-1}**	4.08×10^{-3}	2.07×10^{-1}			
						$6.25-8.33 \times 10^{-3}$	*A*	NMBBR

Ref[1]: A=Falås et al., (2013) (rate constants: L h^{-1} gTS^{-1}); B=Suarez et al., (2010) (rate constants: L h^{-1} $gVSS^{-1}$); C=Plósz et al., (2012) (rate constants: L h^{-1} $gTSS^{-1}$); D=Falås et al., (2012) (rate constants: L h^{-1} gTS^{-1}). DNAS: Denitrifying Activated Sludge; NMBBR: Nitrifying MBBR.

3 Pharmaceuticals in municipal effluent, removed by a laboratory-scale MBBRs

3.1 Influence of humic acid addition on the removal of pharmaceuticals

3.1.1 Experimental procedures

The characteristics of bacteria from biomass in wastewater have been found to be related to and affected by the conditions in which they grow (Cydzik-Kwiatkowska and Zielińska, 2016). Among various wastewater parameters ensuring living conditions for bacterial growth, the concentration and category of the carbon source play a vital role. Mechanisms for the biodegradation of pharmaceuticals in the presence of organic carbon can be classified in two ways: co-metabolism and competitive inhibition.

Co-metabolism involves the transformation of a non-growth substrate (i.e. micropollutants) while a growth substrate (i.e. available carbon source) exists. Previous studies have found that the biodegradation of 4-chlorophenol, considered a non-growth substrate, is enhanced when introducing primary growth substrates, for instance phenol and glucose (Tobajas et al., 2012). However, for competitive inhibition, although the growth substrate is a prerequisite for the degradation of a non-growth substrate, the bacterial enzymes which are responsible for non-growth substrate biodegradation may interact with the growth substrate as a kind of competitor, and thus the biodegradation rate of the non-growth substrate is inhibited and decreases. Joss et al. (2004) found that differences in the removal rates of oestrogens, between the batch experiment and the corresponding compartment of full-scale plants, could be interpreted in terms of the competitive inhibition of oestrogen degradation by the substrate.

In this study, humic acid (HA), considered a not easily degradable carbon source, was used to simulate different concentrations of complex carbon sources in wastewater. A laboratory-scale MBBRs mimicked a polishing step for WWTP effluent. The experiments were performed in Erlenmeyer flasks containing spiked pharmaceuticals, MBBR carriers and WWTP effluent, with or without the addition of HA (blank). A 10 µL pharmaceutical stock solution was transferred to each flask, which had initial concentrations of pharmaceuticals ranging from 1.2 and 14.6 µg/L. MBBR carriers with attached biofilm fed by wastewater effluent for three months were placed into each flask, and

the filling ratios of the carriers and wastewater volume were constant. Three differently defined concentrations of HA were applied and thus gave dissolved organic carbon in quantities of 4.4, 11, 13 and 30 mgC/L. The Erlenmeyer flasks were placed on a mechanical shaker (120 rpm) for a period of two weeks, and samples were taken over time. Details of the methods employed, common wastewater parameters and pharmaceutical analysis are described in paper II.

3.1.2 Influence of humic acid on pharmaceutical degradation

MBBR carriers in each flask had 2.5 g/L of biomass, which was lower than the values observed from previous studies, where around 3.1 g/L was obtained (Falås et al., 2013). This can be explained by the fact that the carriers used in this study were fed by wastewater effluent containing very little carbon utilised for biofilm growth.

The development of pharmaceutical concentrations with different HA dosages over time was plotted by first-order kinetics (Equation **2.1**). All investigated compounds are presented in Figure 3.1. Thirteen out of the 22 detected pharmaceuticals were recognised as being biodegradable by biofilm, because the differences in pharmaceutical concentrations between the controlled experiments (new carriers without biofilm) and the comparative experiments (with attached biofilm) were clear when increasing DOC by adding HA. Specifically, there was low or no activity in relation to the removal of pharmaceuticals in the control experiment compared to the high extent of pharmaceutical removal in the comparative experiments. For the remaining pharmaceuticals (nine out of 22), they were considered to belong to the non-biodegradable group. This definition can be interpreted in two ways. First, several compounds (phenanzone, sulfadiazine, sulfamethoxazole, tramadol and venlafaxine) were not removed in either the controlled or the comparative experiments. For the second aspect, other compounds (azithromycin, clarithromycin, erythromycin, roxithromycin), in relation to the development of pharmaceutical concentrations, did not differ statistically from the controlled or comparative experiments. Therefore, the 13 biodegradable compounds above, which showed the influence of adding HA on the removal of pharmaceuticals, were selected and are discussed in the following sections.

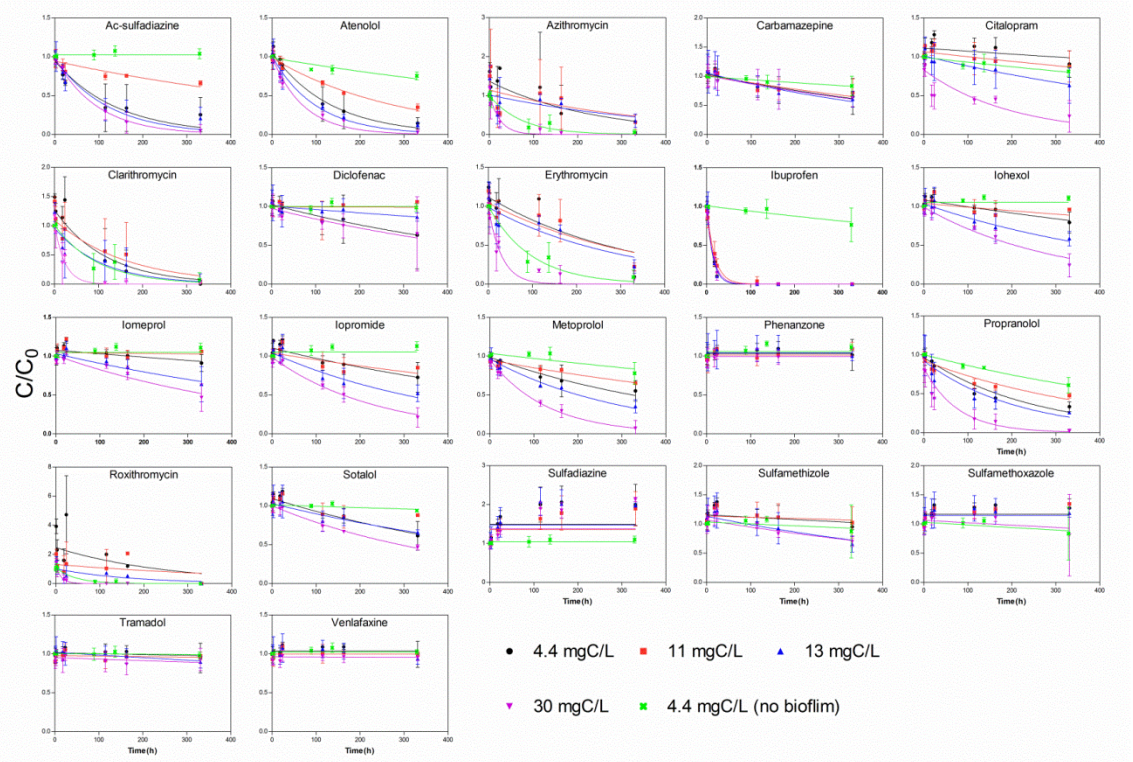

Figure 3.1. Normalised concentration of pharmaceuticals with initial values fitted by first-order kinetics (Equation **2.1**) in batch incubations of MBBR carriers under different initial DOCs. The legend shows the measured initial DOC concentrations. Controls were flasks using new carriers (without biofilm). Error bar indicates standard deviation (**Paper II**).

For these biodegradable compounds, an overview regarding the rate constant of each compound under different DOC concentrations is presented in Figure 3.2. There was a positive correlation between rate constant and DOC values (HA concentration), the higher rate constant along with a higher DOC. Therefore, we can assume co-metabolism acted as the main interaction mechanism between the biodegradation of pharmaceuticals and the addition of HA as an extra carbon source. Otherwise, if a competitive mechanism were involved, the results in Figure 3.2 were opposite. Due to the continuous shaking of the flasks during the experiments, this study replicated aeration conditions. Tran et. al (2013) also found pharmaceuticals were oxidised during the microbial metabolism of another growth substrate in aeration conditions.

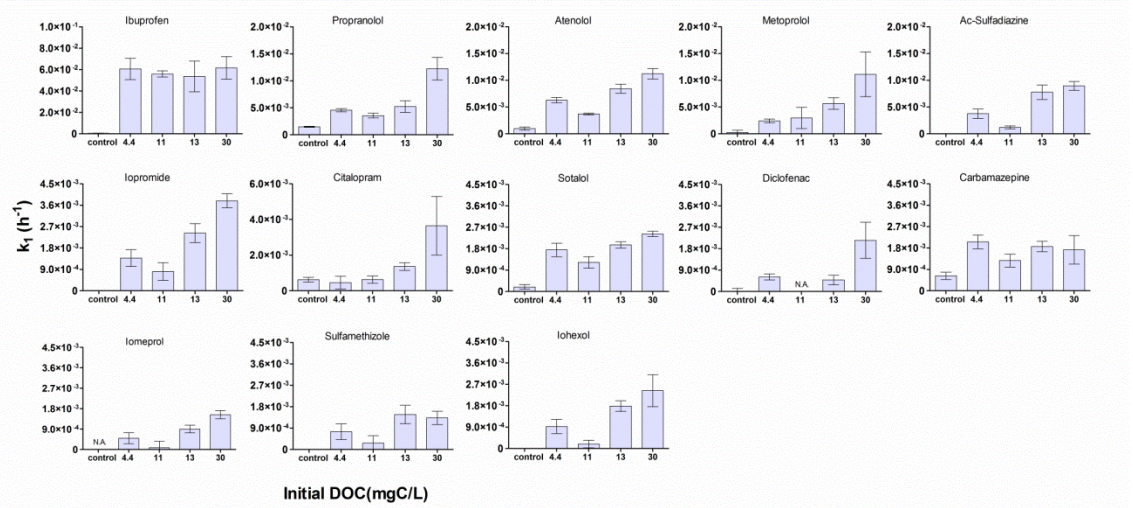

Figure 3.2. Rate constants (k) of pharmaceuticals in the batch incubations of MBBR carriers under different initial DOCs. Control stands for flasks containing carriers without biofilm. Control flasks contained also 4.4 mgC/L of DOC. N.A. indicates that the concentration curve did not fit to Equation **2.1**. Error bar indicates standard deviation (**Paper II**).

3.1.3 Correlation of total concentration of organic matter and rate constant

The statistical results for the correlation of the rate constants of 13 biodegradable pharmaceuticals, and the initial DOC values, are presented in Table 3.1, based on the first-order kinetics fitting results taken from Figure 3.2. Ten out of the 13 compounds (except carbamazepine, ibuprofen and sulfamethizole) had high or very high R^2, indicating that the addition of HA had stimulated the pharmaceutical biodegradation. Calculating the difference of rate constants in high DOC (30 mgC/L) and low DOC (4.4 mgC/L), normalised with the relevant DOC difference, the average stimulation for all biodegradable pharmaceuticals was 7.4% per mgC/L, and the 25%, 50% and 75% percentiles were 2.8, 6.4 and 8.7% per mgC/L, respectively (Figure 3.3). Besides, the values of the rate constant in high DOC normally were two or three times higher than the values obtained from the controlled experiment (without biofilm).

Table 3.1. Removal rate constant, ki, and the correlation between rate constants and initial DOC. The correlation between the parameters expressed as R^2 is categorised as VH (very high) H (high correlation) M (moderate correlation and L (low correlation) according to the criteria in Asuero et al. (2006) (**Paper II**).

Compound	4.4 mgC/L DOC	11 mgC/L DOC	13 mgC/L DOC	30 mgC/L DOC	Rate constant vs DOC		
	k_{HA0} (h^{-1})	k_{HA1} (h^{-1})	k_{HA2} (h^{-1})	k_{HA3} (h^{-1})	Slope ($L \cdot mgC^{-1} \cdot h^{-1}$)	R^2	Corr.
Ac-Sulfadiazine	$3.8\pm0.9\cdot10^{-3}$	$1.2\pm0.3\cdot10^{-3}$	$8\pm1\cdot10^{-3}$	$9\pm0.8\cdot10^{-3}$	$2.3\cdot10^{-4}$	0.50	H
Atenolol	$6.3\pm0.5\cdot10^{-3}$	$3.7\pm0.2\cdot10^{-3}$	$8.4\pm0.8\cdot10^{-3}$	$11.2\pm1\cdot10^{-3}$	$2.3\cdot10^{-4}$	0.61	H
Carba-mazepine	$2.1\pm0.3\cdot10^{-3}$	$1.3\pm0.3\cdot10^{-3}$	$1.9\pm2\cdot10^{-3}$	$1.7\pm0.6\cdot10^{-3}$	$(-)5.6\cdot10^{-6}$	0.03	L
Citalopram	$4.6\pm3.6\cdot10^{-4}$	$6.4\pm2.1\cdot10^{-4}$	$1.4\pm0.2\cdot10^{-3}$	$3.7\pm1.6\cdot10^{-3}$	$1.31\cdot10^{-4}$	0.96	VH
Diclofenac	$0.6\pm0.1\cdot10^{-3}$	$1.0\cdot10^{-16}$	$5.0\pm2\cdot10^{-3}$	$2.2\pm0.8\cdot10^{-3}$	$0.7\cdot10^{-4}$	0.74	H
Ibuprofen	$61\pm10\cdot10^{-3}$	$56\pm3\cdot10^{-3}$	$50\pm1\cdot10^{-3}$	$60\pm10\cdot10^{-3}$	$1.2\cdot10^{-4}$	0.12	L
Iohexol	$0.9\pm0.3\cdot10^{-3}$	$0.2\pm0.2\cdot10^{-3}$	$1.8\pm0.2\cdot10^{-3}$	$2.4\pm0.7\cdot10^{-3}$	$0.7\cdot10^{-4}$	0.58	H
Iomeprol	$0.5\pm0.2\cdot10^{-3}$	$0.1\pm0.3\cdot10^{-3}$	$0.9\pm0.2\cdot10^{-3}$	$1.5\pm0.2\cdot10^{-3}$	$0.5\cdot10^{-4}$	0.69	H
Iopromide	$1.4\pm0.4\cdot10^{-3}$	$0.8\pm0.4\cdot10^{-3}$	$2.4\pm0.4\cdot10^{-3}$	$3.8\pm0.3\cdot10^{-3}$	$1.1\cdot10^{-4}$	0.77	H
Metoprolol	$2.4\pm0.4\cdot10^{-3}$	$3.0\pm2\cdot10^{-3}$	$6\pm1\cdot10^{-3}$	$11\pm4\cdot10^{-3}$	$3.6\cdot10^{-4}$	0.95	VH
Propranolol	$4.6\pm0.3\cdot0^{-3}$	$3.6\pm0.4\cdot10^{-3}$	$5\pm1\cdot10^{-3}$	$12\pm2\cdot10^{-3}$	$3.4\cdot10^{-4}$	0.87	VH
Sotalol	$1.8\pm0.3\cdot10^{-3}$	$1.2\pm0.2\cdot10^{-3}$	$2\pm0.1\cdot10^{-3}$	$2.4\pm0.1\cdot10^{-3}$	$0.3\cdot10^{-4}$	0.54	H
Sulfa-methizole	$0.8\pm0.3\cdot10^{-3}$	$0.3\pm0.3\cdot10^{-3}$	$1.5\pm0.4\cdot10^{-3}$	$1.3\pm0.3\cdot10^{-3}$	$0.3\cdot10^{-4}$	0.28	M

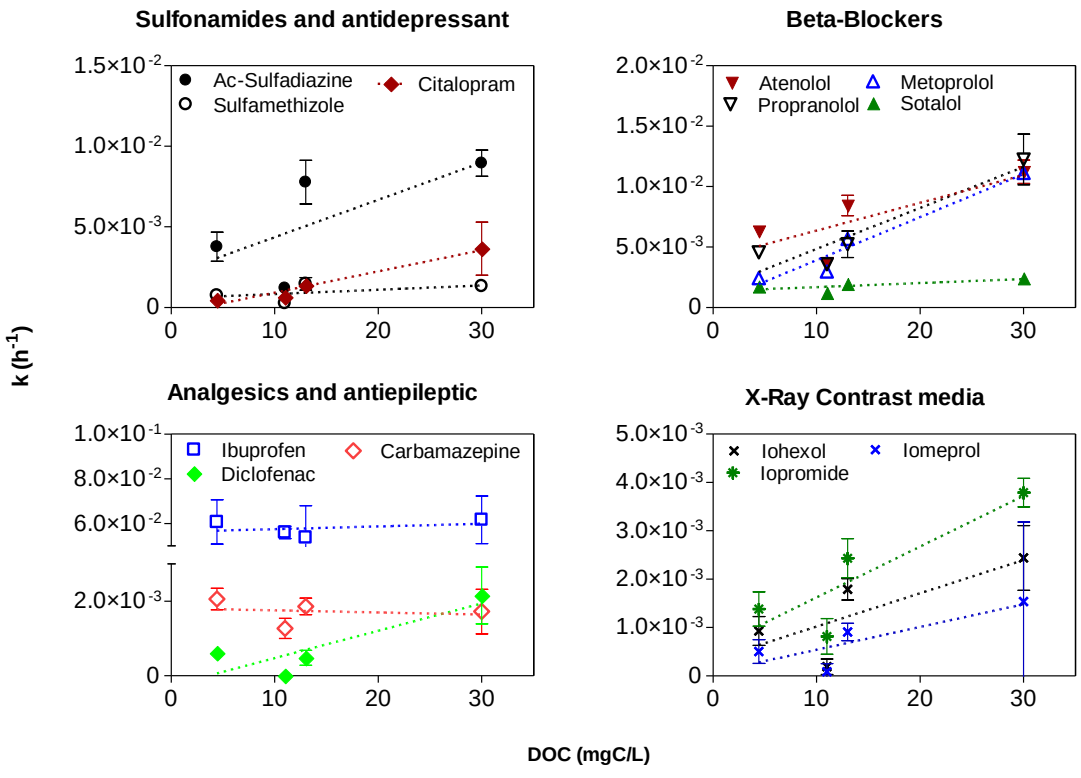

Figure 3.3. Plot of all the removal rate constants (k) obtained by single-fitting first-order kinetics (Equation **2.1**) to the concentrations of all biodegradable pharmaceuticals in the batch incubations of MBBR carriers versus different initial DOCs. Error bar indicates standard deviation (**Paper II**).

3.2 Removal of pharmaceuticals by an intermittently fed polishing MBBRs

3.2.1 MBBRs system and experimental methods

Since conventional WWTPS (i.e. activated sludge) are not able to remove all micropollutants from wastewater, some micropollutants, such as pharmaceuticals, are well-known as persistent compounds that remain detectable in WWTP effluent (Verlicchi et al., 2012a, 2012b). Therefore, polishing treatments for WWTP effluent need be carried out to upgrade and improve the quality of effluent, in order to achieve discharge standards. In previous studies, MBBRs have been used to demonstrate that most pharmaceuticals can be degraded to some extent and with better removal efficiency compared to activated sludge (Escolà Casas et al., 2015a). However, for refractor compounds (i.e. diclofenac), their remove rates are still low and result in poor water quality. For diclofenac, as one of three crucial compounds in the first watch list authority set up by the European Commission (Carvalho et al., 2015), its degradation rate when treated with current wastewater purification processes is not optimistic. Thus, it is an urgent undertaking to find a solution to this issue.

A previous MBBR study was conducted by three pilot-scale static-staged MBBRs fed by raw hospital wastewater (Escolà Casas et al., 2015a). Hence, the first reactor in this treatment train had more nutrients for biofilm growth compared to the two following reactors. The last reactor particularly had to utilise hardly degradable nutrients from the second reactor's effluent, to support biofilm activity, where mostly the thinnest biofilm was observed in the last reactor as well as the thickness of biofilm or the amount of biomass decay from the first reactor to the last reactor. Based on k_{bio} of diclofenac in the last reactor from a former MBBR study, although the removal degree of diclofenac was low, this reactor degrader was capable of degrading diclofenac did exist, and the reason for ineffective removal could be explained by a lack of biomass. Then, to improve the overall removal of diclofenac by staged MBBRs, the removal control in the last reactor was very important, since this reactor acted as the last line of defence against pharmaceuticals before discharging the effluent. To elevate the amount of degrader in the last reactor, the primary task is to achieve an amount of biomass sufficient enough to eventually benefit degrader growth.

Thus, in this study, MBBRs were used for polishing wastewater effluent from a conventional WWTP in Denmark and to overcome inactive biomass generation in the last reactor, due to low substrate concentration. For the first time, we carried out a programme of intermittent feeding to MBBR reactors with raw wastewater from a primary settling tank, and WWTP effluent. The description for the above feeding strategy is illustrated in Figure 3.4. Essentially, three identical 3 L reactors with 50% filling ratio of carriers were used. A two-stage MBBR treatment train (reactors 1 and 2 in positions A and B; Figure 3.4a) was fed with CAS effluent and performed as a polishing reactor. Another MBBR treatment train with a single reactor (reactor 3 in position C) was fed with settled raw wastewater, which we used to stimulate biomass generation as a regenerated reactor. After two days of operation, the feeding of reactors in these two MBBR treatment trains was changed, and thus reactor 3 was placed in position A and fed with CAS effluent, reactor 1 was then moved to Position B and reactor 2 was switched to position C to operate as a regeneration reactor (Figure 3.4b). Following a further two days, the feeding programme was changed again, as outlined above. This was followed by another three days of operation before changing the next feeding regime so that the reactors returned back to their initial positions, as presented in Figure 3.4a. This feeding strategy was conducted three times per week.

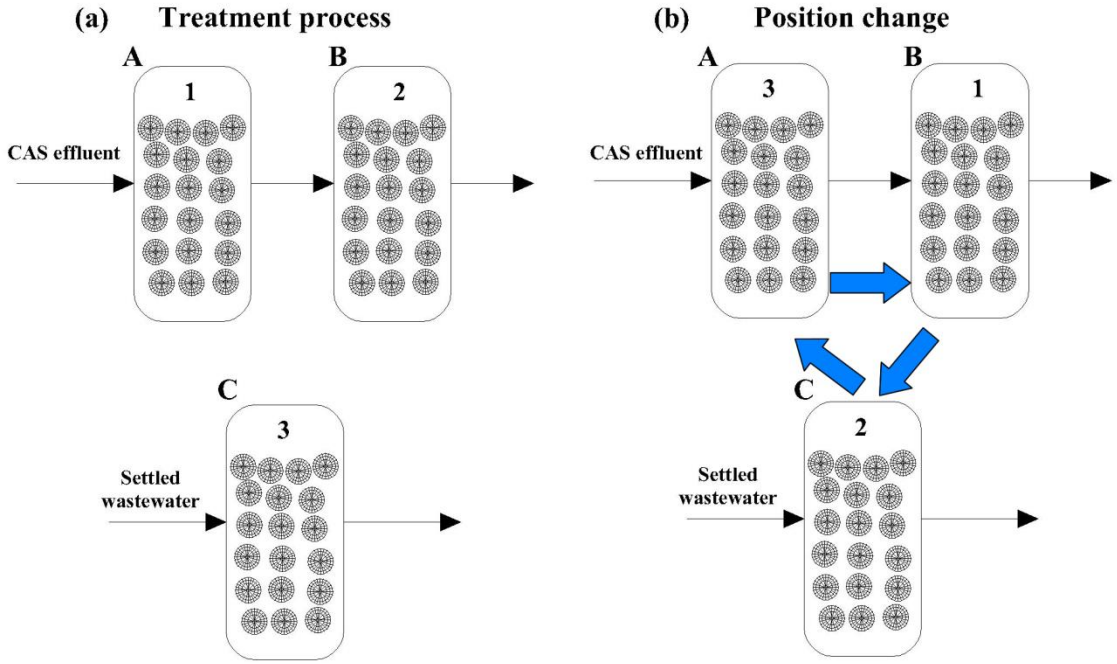

Figure 3.4. Configuration of the MBBR system: (a) Operation of a two-stage MBBR treatment train polishing effluent water from the Viby WWTP (positions A and B), while the growth of biofilm was stimulated in another MBBR treatment train with a single reac-

tor (position C). (b) The change of feeding to two MBBR treatment trains was conducted three times per week. (**Paper III**)

3.2.2 Performance of intermittently fed MBBRs on wastewater parameters

To ensure the non-static polishing MBBR system remained stable, common wastewater parameters were measured during the four-month operating period (Table 3.2).

Table 3.2. Common wastewater parameters of reactors in three positions in an MBBR over a long time period (13/04/2015-24/08/2015). Indicated intervals (±) are standard deviation of means. DO: Dissolved oxygen, DOC: dissolved organic carbon. (**Paper III**)

Reactor	HRT (h)	pH	DO ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	NH_4^+-N ($mg \cdot L^{-1}$)
CAS effluent		7.4±0.1		8.2±1.3	0.84±0.44
Position A	0.5	7.7±0.5	7.2±0.9	8.1±1.2	0.16±0.10
Position B	0.5	8.0±0.5	8.3±0.9	8.7±2.1	0.04±0.04
Settled raw wastewater		7.6±0.1		22±5.0	24±5
Position C		7.8±0.5	7.4±1.4	9.1±1.6	0.28±0.16

'CAS effluent' represents effluents from the full-scale WWTP, and 'settled raw wastewater' stands for wastewater taken from the primary settler which was used for feeding the reactor in position C. In this intermittently fed system, concentrations of NH_4^+-N from influent to effluent were almost removed totally, which demonstrates this MBBR system had good nitrification ability. This phenomenon, on the other hand, is also supported by the dissolved oxygen concentration (DO) in each reactor at above 2 $mg \cdot L^{-1}$, which was required for processing aeration reactions. The average biomass in positions A, B and C during the period 13/04/15 to 24/08/2015 were 1.3±0.2, 1.1±0.2 and 1.0±0.2 $g \cdot L^{-1}$, respectively. Therefore, biomass was considered identical in all three reactors, which agrees with the fact that there is not enough time in positions A and B to lose significant amounts of biomass, due to the rapid changing of the reactor feed patterns.

3.2.3 Biodegradation of pharmaceuticals in the MBBRs

To test the potential capacity of pharmaceutical removal by intermittently fed MBBR systems, a batch experiment was conducted. During this experiment, the water flow of influent and in between reactors was stopped and a stock solution of pharmaceuticals was spiked into each reactor, which gave the initial concentration of pharmaceuticals at around 3-20 $\mu g \cdot L^{-1}$ (Figure 3.5a). Then, samples were taken from each reactor over time. However, to test the actual performance of intermittently fed MBBR systems in real life, a contin-

uous flow experiment was conducted. In this experiment, the water flow was allowed to travel through the systems without stopping or spiking (Figure 3.5b). Samples were taken from the influent and reactor effluents according to hydraulic retention time (HRT).

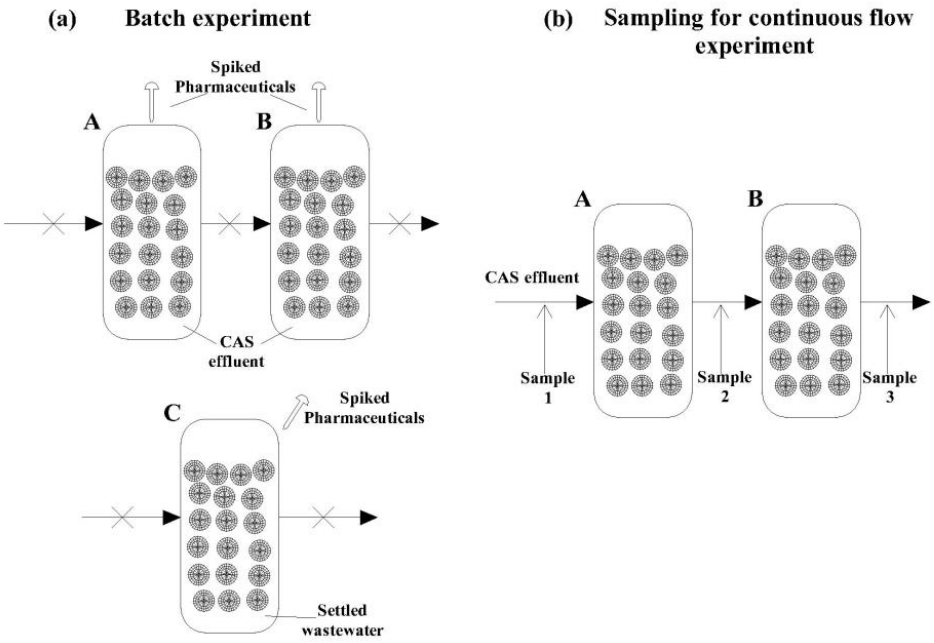

Figure 3.5. Configuration of the staged MBBR during the batch and continuous flow experiments: (a) the batch experiments were carried out to measure the biofilms' capacity to degrade pharmaceuticals, and the flow was discontinued while concentrations of spiked pharmaceuticals were measured over time. (b) During the continuous flow experiment. (**Paper III**)

For the batch experiment, pharmaceutical concentrations over time were plotted and fitted with first-order kinetics and are presented in Figure 3.6. Applying first-order kinetics to describe the development of pharmaceutical concentration was feasible, since most of the pharmaceuticals were able to obtain good R^2 values (>0.96), except for propranolol, tramadol and venlafaxine, and similar findings were also observed in a previous study (Escolà Casas et al., 2015b). In Figure 3.6, all of the investigated pharmaceuticals, except carbamazepine, defined as a recalcitrantly biodegradable compound (Joss et al., 2006), demonstrated the potential to be biodegradable to some degree. In terms of diclofenac, its half-life was around 2.1 h, and within 12 h it could be removed entirely, which was faster than any biological treatment processes examined so far.

Acetyl-sulfadiazine is a conjugation product formed through the human metabolism of sulfadiazine; however, rapid de-conjugation usually occurs through relevant microorganisms in wastewater, which eventually transfer back to sulfadiazine again. Therefore, in this study, in the first five hours of reaction, the concentration of sulfadiazine increased due to the contribution of the de-conjugation of ac-sulfadiazine, and so the concentration of ac-sulfadiazine decreased and moved gradually closer to zero within the first five hours. After five hours, sulfadiazine concentration started to decrease until the last sampling time, while ac-sulfadiazine was barely detected and therefore no longer contributed. Kovalova et al. (2012) also found the similar reactions for other compounds in the sulphonamides group.

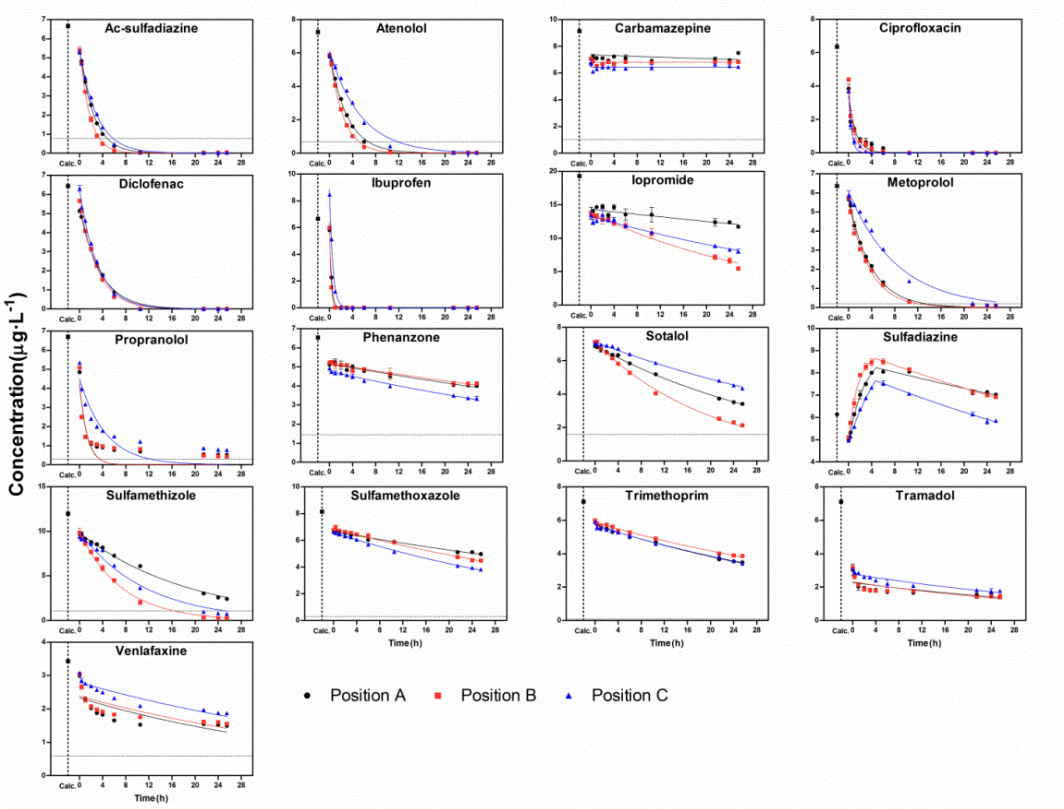

Figure 3.6. First-order reaction rate fitting to concentrations of pharmaceuticals (except sulfadiazine) in batch experiments (reactor in position A, fed by CAS effluent, and then effluent from position A flows into the reactor in position B. However, the reactor in position C is fed by settled raw wastewater. Before starting the spiking experiment, the flow of each reactor was stopped). The dashed horizontal line stands for the limit of quantification (LOQ), as derived from the lower of two multiple reaction monitoring (MRM) signals. (**Paper III**)

In Table 3.3, the rate constant (k) and k_{bio} of pharmaceuticals in this study are compared to three similar MBBR studies. For the k_{bio} of intermittently fed

reactors in this study, eight out of 15 pharmaceuticals (i.e. atenolol, ciprofloxacin, diclofenac, iopromide, metoprolol, sulfamethizole, tramadol and venlafaxine) for reactors in positions A and B increased in comparison to the static staged reactors (Escolà Casas et al., 2015a, 2015b). In terms of diclofenac, k_{bio} in this study was ten times higher or even more than its values obtained from previous researches. Thus, the amount of degrader response to degrade diclofenac was promoted through the novel intermittent feeding concept.

Table 3.3 Comparison of rate constants (k, h^{-1}) and biomass normalised rate constants (k_{bio}, $L \cdot h^{-1} \cdot g^{-1}$). (**Paper III**)

	k (h^{-1})			k_{bio} ($L \cdot h^{-1} \cdot g^{-1}$)							
	This experiment			Effluent polishing of suspended biofilm (This experiment)			Staged MBBR WWTP [a]			Effluent polishing MBBR [b]	Biofilm in IFAS WWTP [c]
Compound	Position A	Position B	Position C	Position A	Position B	Position C	Stage 1	Stage 2	Stage 3		
Ac-Sulfadiazine	$41 \pm 1 \cdot 10^{-2}$	$57 \pm 1 \cdot 10^{-2}$	$33 \pm 1 \cdot 10^{-2}$	$34 \cdot 10^{-2}$	$39 \cdot 10^{-2}$	$24 \cdot 10^{-2}$	$35 \cdot 10^{-2}$	$77 \cdot 10^{-2}$	$3.8 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	
Atenolol	$32 \pm 1 \cdot 10^{-2}$	$43 \pm 1 \cdot 10^{-2}$	$19 \pm 1 \cdot 10^{-2}$	$26 \cdot 10^{-2}$	$29 \cdot 10^{-2}$	$13 \cdot 10^{-2}$	$8.2 \cdot 10^{-2}$	$18 \cdot 10^{-2}$	$14 \cdot 10^{-2}$	$5.0 \cdot 10^{-2}$	$2.5 \cdot 10^{-2}$
Ciprofloxacin	$79 \pm 17 \cdot 10^{-2}$	$119 \pm 19 \cdot 10^{-2}$	$201 \pm 22 \cdot 10^{-2}$	$65 \cdot 10^{-2}$	$80 \cdot 10^{-2}$	$146 \cdot 10^{-2}$	$0.3 \cdot 10^{-2}$	$0.8 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$		
Diclofenac	$28 \pm 1 \cdot 10^{-2}$	$33 \pm 1 \cdot 10^{-2}$	$32 \pm 1 \cdot 10^{-2}$	$23 \cdot 10^{-2}$	$22 \cdot 10^{-2}$	$23 \cdot 10^{-2}$	$2.6 \cdot 10^{-2}$	$5.7 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$	$0.3 \cdot 10^{-2}$	$6.3 \cdot 10^{-2}$
Ibuprofen	$313 \pm 13 \cdot 10^{-2}$	$433 \pm 6 \cdot 10^{-2}$	$181 \pm 7 \cdot 10^{-2}$	$258 \cdot 10^{-2}$	$291 \cdot 10^{-2}$	$131 \cdot 10^{-2}$	$131 \cdot 10^{-2}$	$291 \cdot 10^{-2}$	$48 \cdot 10^{-2}$		
Iopromide	$0.7 \pm 0.1 \cdot 10^{-2}$	$3.1 \pm 0.2 \cdot 10^{-2}$	$1.9 \pm 0.1 \cdot 10^{-2}$	$0.6 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$	$0.3 \cdot 10^{-2}$	$0.7 \cdot 10^{-2}$	$2.0 \cdot 10^{-2}$	$0.7 \cdot 10^{-2}$	
Metoprolol	$25 \pm 1 \cdot 10^{-2}$	$28 \pm 1 \cdot 10^{-2}$	$12 \pm 1 \cdot 10^{-2}$	$21 \cdot 10^{-2}$	$19 \cdot 10^{-2}$	$8.7 \cdot 10^{-2}$	$2.3 \cdot 10^{-2}$	$5.2 \cdot 10^{-2}$	$3.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$
Phenanzone	$1.1 \pm 0.1 \cdot 10^{-2}$	$1.0 \pm 0.1 \cdot 10^{-2}$	$1.5 \pm 0.1 \cdot 10^{-2}$	$0.9 \cdot 10^{-2}$	$0.7 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$0.9 \cdot 10^{-2}$	$1.9 \cdot 10^{-2}$	$3.6 \cdot 10^{-2}$	$0.6 \cdot 10^{-2}$	$0.4 \cdot 10^{-2}$
Propranolol	$77 \pm 24 \cdot 10^{-2}$	$77 \pm 26 \cdot 10^{-2}$	$22 \pm 6 \cdot 10^{-2}$	$64 \cdot 10^{-2}$	$52 \cdot 10^{-2}$	$16 \cdot 10^{-2}$	$76 \cdot 10^{-2}$	$169 \cdot 10^{-2}$	$13 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$	
Sotalol	$2.8 \pm 0.1 \cdot 10^{-2}$	$4.9 \pm 0.1 \cdot 10^{-2}$	$1.9 \pm 0.1 \cdot 10^{-2}$	$2.3 \cdot 10^{-2}$	$3.3 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$	$2.6 \cdot 10^{-2}$	$5.8 \cdot 10^{-2}$	$3.1 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$	
Sulfamethizole	$5.3 \pm 0.2 \cdot 10^{-2}$	$14 \pm 1 \cdot 10^{-2}$	$8.8 \pm 0.7 \cdot 10^{-2}$	$4.3 \cdot 10^{-2}$	$9.2 \cdot 10^{-2}$	$6.4 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$	$2.1 \cdot 10^{-2}$	$2.9 \cdot 10^{-2}$	$0.9 \cdot 10^{-2}$	
Sulfamethoxazole	$1.2 \pm 0.0 \cdot 10^{-2}$	$1.7 \pm 0.1 \cdot 10^{-2}$	$2.2 \pm 0.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$1.6 \cdot 10^{-2}$	$0.8 \cdot 10^{-2}$	$1.8 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$0.4 \cdot 10^{-2}$	
Tramadol	$2.0 \pm 1.0 \cdot 10^{-2}$	$2.2 \pm 0.6 \cdot 10^{-2}$	$2.0 \pm 0.2 \cdot 10^{-2}$	$1.6 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$	$0.4 \cdot 10^{-2}$	$0.8 \cdot 10^{-2}$	$0.5 \cdot 10^{-2}$	$0.6 \cdot 10^{-2}$	
Trimethoprim	$2.0 \pm 0.1 \cdot 10^{-2}$	$1.7 \pm 0.1 \cdot 10^{-2}$	$2.0 \pm 0.1 \cdot 10^{-2}$	$1.7 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$	$2.8 \cdot 10^{-2}$	$6.3 \cdot 10^{-2}$	$2.9 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$9.0 \cdot 10^{-2}$
Venlafaxine	$2.3 \pm 0.7 \cdot 10^{-2}$	$2.0 \pm 0.6 \cdot 10^{-2}$	$1.8 \pm 0.2 \cdot 10^{-2}$	$1.9 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$	$1.3 \cdot 10^{-2}$	$0.4 \cdot 10^{-2}$	$0.9 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$	$0.6 \cdot 10^{-2}$	$0.4 \cdot 10^{-2}$

(a): a three-stage MBBR system (reactors 1, 2 and 3) fed by wastewater from the oncology section of Aarhus University Hospital (Escolà Casas et al., 2015a).
(b): one-stage MBBR (reactor H4) which was a polishing process after treatment with activated sludge combined with MBBR (Hybas) (also fed by wastewater from the oncology section of Århus University Hospital) (Escolà Casas et al., 2015b).
(c): One-stage IFAS (integrated fixed-film activated sludge, reactor M) stands for a 10 L reactor filled with wastewater and carriers from a domestic WWTP in Switzerland (Falås et al., 2013).

Additionally, similar results can also be found for sulfamethizole (an antibiotic). The k_{bio} in positions A and B, especially position B, was significantly higher than in the three reactors from the statically staged MBBR (Escolà Casas et al., 2015a).

For continuous flow experiments, natural concentrations of pharmaceuticals without spiking were analysed in influent and the reactors' effluent (Figure 3.7). In reality, diclofenac concentration decreased from influent to effluent, which in turn confirmed the potential biodegradability seen in the batch experiment. Furthermore, the removal of pharmaceuticals increased in line with an increase in HRT; for instance, atenolol was totally degraded when HRT increased from 1 h to 4 h, and the removal of metoprolol increased from 7% to 69%, while the removal of propranolol increased from 3% to 43%. For sulfamethizole, removal changed from negative to 48 %.

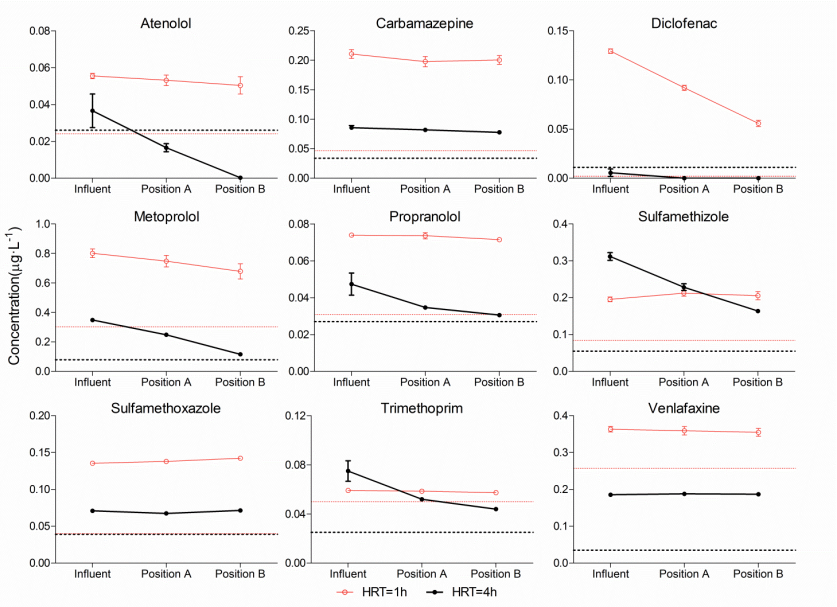

Figure 3.7. Concentrations of selected pharmaceuticals in continuous experiments with different HRTs. The dashed horizontal lines indicate the LOQ for each pharmaceutical, derived from two multiple reaction monitoring (MRM) transitions. (**Paper III**)

4 Pharmaceuticals in the effluent of a pilot-scale staged MBBRs treated by ozone and followed with a polishing MBBR

4.1 MBBRs/ozone system and experimental methods

Although MBBRs are better at removing pharmaceuticals compared to CAS, and thus may be considered as an alternative for conventional treatment processes applied in most WWTPs, some hardly biodegradable pharmaceuticals were still be detected in MBBRs effluents, as mentioned in Chapter 2, such as X-ray contrast medias, venlafaxine, carbamazepine and so on.

To address this issue, ozone, known with matured implement experiences to balance the sufficient removal of pharmaceuticals and operation expenses, is a feasible technology, using as a polishing method to enhance pharmaceutical removal (Hansen et al., 2016; Ternes et al., 2003). The oxidation of pharmaceuticals by ozonation results in two vital paths: a direct reaction with certain functional groups of organic molecules (**4.1**) and an indirect/non-selective reaction with strong oxidant hydroxyl radicals (**4.2**) (Dantas et al., 2007).

$$R + O_3 \rightarrow R_{OX} \qquad (\mathbf{4.1})$$

$$R + OH\cdot \rightarrow R_{OX} \qquad (\mathbf{4.2})$$

In this study, pilot-scale ozone setups, mainly consisting of an ozone generator and an ozone reaction column, were applied for polishing effluents from two pilot-scale staged MBBRs receiving either raw hospital wastewater (Skejby, Denmark) or municipal wastewater (Herning, Denmark), as described in Chapter 2 as well. The HRT for the ozone setup connected with the staged MBBR treating hospital wastewater was 13.1 min, based on a 1 L/min flow rate of ozone influent and a 13 L column volume. A schematic diagram of the treatment configuration can be found in Figure 2.1(right).

However, the HRT for the ozone setup connected with the staged MBBR treating municipal wastewater was 9 min, according to a 2 L/min influent flow rate and an 18 L reactor. Furthermore, in order to purify and reduce further the effluent toxicity generated from ozone by-products, a pilot-scale

MBBR with a HRT of 14 min was applied for polishing ozonation effluent. A description of the treatment configuration is illustrated in Figure 2.2 (right).

For performance tests of both of the polishing methods above, first, to optimise the dosage of ozone to obtain high pharmaceutical removal efficiency, different doses were applied into the reaction column. Furthermore, to verify pharmaceutical removal by ozonation, the same MBBR effluents were taken back to the laboratory and ozonation experiments carried out in a laboratory-scale ozone setup with similar ozone doses. Meanwhile, the fluorescence intensity of both ozonated wastewaters in the pilot and laboratory tests was measured under six selected wavelengths. Second, toxicity development in the wastewater in line with the treatment configurations in the pilot were measured, and a toxicity test of MBBRs effluents treated with laboratory-scale ozonation followed by a polishing MBBR were also conducted.

To determine the ozone dosage that achieved 90% removal of each pharmaceutical in the effluent, the correlation of degradation rate of each pharmaceutical and ozone dosage was fitted by equation (**4.3**).

$$\frac{C}{C_0} = 10^{-(\frac{DO_3}{DDO_3})} \qquad (\mathbf{4.3})$$

The remaining concentration of pharmaceutical (C) is related to its initial concentration (C_0) after a relevant reaction with a specific delivered ozone dose (DO_3) with a decadic dose of ozone (DDO_3), as the compound-specific constant describing the required ozone dosage needed to remove 90% of the respective pharmaceutical. This was based on the fact that that the decay of ozone is determined by the effluent matrix and is independent of pharmaceutical concentration.

4.2 Removal of pharmaceuticals

Normalised concentrations of pharmaceuticals with different doses of ozone were plotted according to the above equation (**4.3**). Twenty-two pharmaceuticals were detected in the effluent of the pre-treating MBBRs for hospital wastewater without spiking, while 15 out of 22 compounds were above LOQs (Figure 4.1). However, for effluents from the pre-treating MBBRs for municipal wastewater, 24 pharmaceuticals were detected, while 21 out of 24 compounds were above relevant LOQs (Figure 4.2).

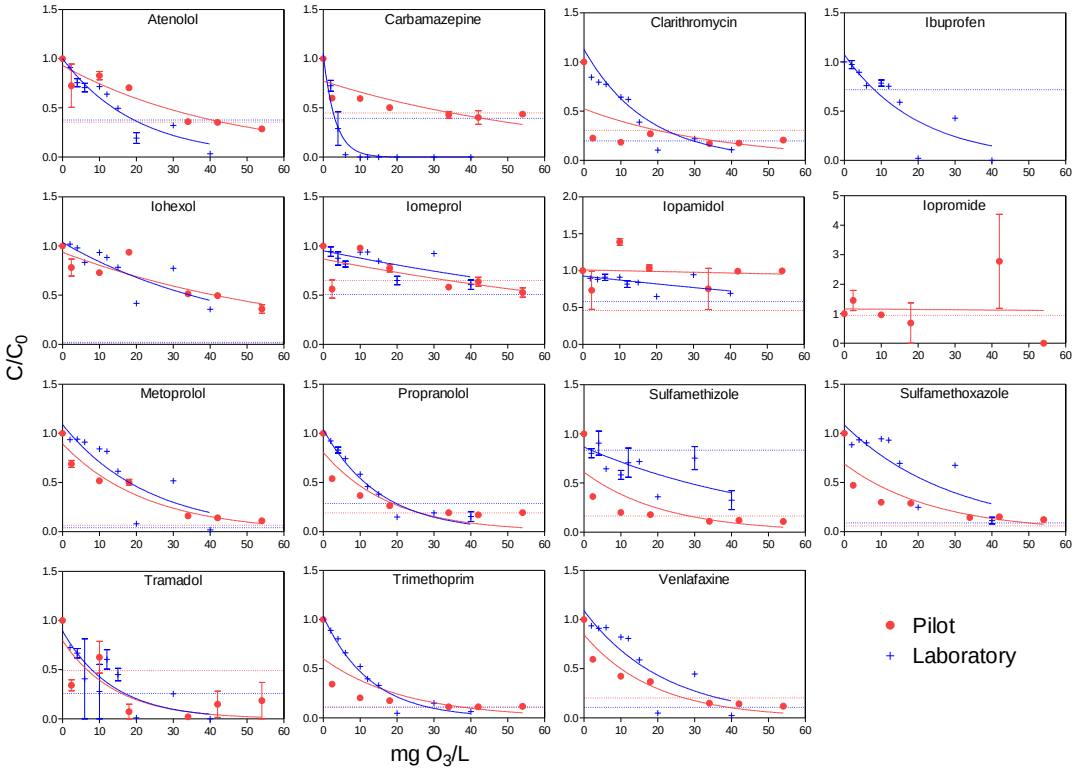

Figure 4.1. Comparison of pharmaceutical removal by ozone in the effluent of the staged MBBR demonstration plant at Skejby Hospital, using the onsite continuous pilot ozonation system and batch treatment in the laboratory. The dashed lines in the figure stand for the limit of quantification (LOQ) of pharmaceuticals by HPLC-MS/MS. Error bars represent standard deviations. (**Paper IV**)

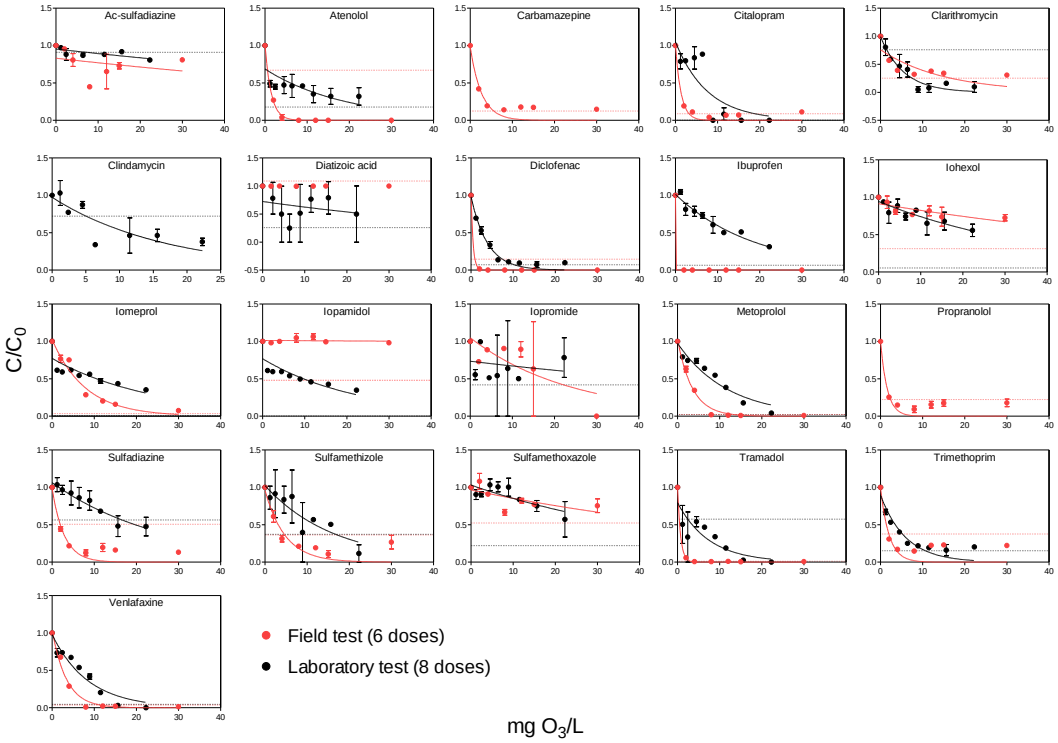

Figure 4.2. Comparison of pharmaceutical removal by ozone in the effluent of the staged MBBR demonstration plant at the Herning municipal treatment plant, using the onsite continuous pilot ozonation system and batch treatment in the laboratory. The dashed lines in the figure stand for the limit of quantification (LOQ) of pharmaceuticals by HPLC-MS/MS. Error bars represent standard deviations. (**Paper V**)

In general, concentrations of pharmaceuticals above LOQs decreased in line with an increase in ozone dose in both MBBR effluent-polished studies. Nonetheless, iohexol, iopamidol and iopromide from the contrast media group were barely removed, even with high ozone concentrations. To evaluate pharmaceutical removal efficiency, the DDO_3 of individual pharmaceuticals was obtained, based on the fitting curve established from equation (**4.3**). When comparing the DDO_3 of each pharmaceutical from one pilot experiment to another, or one laboratory experiment to another or between pilot and laboratory experiments, they were not identical. Many factors could explain these different results, for instance HRT, ozone setups, wastewater substrate and so on. Among these factors, DOC as a critical water substrate parameter is able to affect significantly the performance of the ozonation process (Blaney, 2014). Thus, to make the results of this study more accurate and comparable with other similar studies, the DDO_3 of each pharmaceutical was normalised to effluent DOC and thus Z_{90} was calculated (Table 4.1).

Table 4.1. Ozone dosage for 90% removal of pharmaceuticals in the pilot and laboratory and the normalisation of ozone dosage to the relevant DOC condition (Z_{90}=DDO_3/DOC, DOC of this study is 40 mg O_3/L). Indicated intervals represent one standard deviation. (**Paper IV**)

	Pilot		Laboratory		Z_{90}			
	DDO_3	R^2	DDO_3	R^2	Pilot	Laboratory	A	B
Ac-sulfadiazine	<LOQ[a]	<LOQ						
Atenolol	103±19	0.89	46±7	0.91	2.6±0.5	1.1±0.2		1.1
characterizeAzithromycin	<LOQ	<LOQ	<LOQ	<LOQ				
Carbamazepine	*147±53*[c]	0.63	7.4±1.1	0.96	*3.7±1.3*	0.18±0.03	0.61	0.58
Ciprofloxacin	<LOQ	<LOQ	<LOQ	<LOQ				
Clarithromycin	<LOQ	<LOQ	39±6	0.92		0.98±0.14		0.75
Diatrizoic acid	<LOQ	<LOQ	<LOQ	<LOQ				4.7
Ibuprofen	<LOQ	<LOQ	46±8	0.78		1.1±0.2	1.61	1.3
Iohexol	151±42	0.77	*110±30*	0.69	3.8±1.0	*2.7±0.7*		1.8
Iomeprol	No fit[b]	0.41	No fit	0.44				1.9
Iopamidol	No fit	0.01	No fit	0.34				2.6
Iopromide	<LOQ	<LOQ						
Metoprolol	52±6	0.94	54±10	0.75	1.3±0.2	1.3±0.2	1	0.89
Phenazone	<LOQ	<LOQ	<LOQ	<LOQ				0.77
Propranolol	42±9	0.79	35±2	0.96	1.0±0.2	0.88±0.06		0.6
Sotalol	<LOQ	<LOQ	<LOQ	<LOQ				
Sulfadiazine	<LOQ	<LOQ						0.5
Sulfamethizole	50±12	0.862	<LOQ	<LOQ	1.2±0.03		0.77	0.52
Sulfamethoxazole	56±9	0.74	*68±14*	0.66	1.4±0.2	*1.7±0.3*		0.52
Tramadol	*33±19*	0.64	31±9	0.77	*0.81±0.47*	0.8±0.2		0.97
Trimethoprim	*51±12*	0.61	29±2	0.96	*1.3±0.3*	0.73±0.06	0.55	0.55
Venlafaxine	44±7	0.90	50±9	0.77	1.1±0.2	1.2±0.2	0.91	1.4

[a]: If concentration is below the limit of quantification (LOQ), it indicates <LOQ.

[b]: If R^2 <0.5, it indicates no fit.

[c]: If 0.5<R^2 <0.7, it is considered a poor fit and indicates *Italic*.

A indicates the reference (Antoniou et al., 2013).

B indicates the reference (Hansen et al., 2016).

Antoniou et al. (2013) investigated the influence of different effluent matrices on the removal of spiked pharmaceuticals through laboratory ozonation, while Hansen et al. (2016) studied the effect of DOC on the removal of spiked pharmaceuticals in effluent from a staged MBBR with laboratory ozone equipment, finding that the obtained Z_{90} values from effluent with different DOCs were comparable. When comparing the Z_{90} of the current two studies with previous similar researches, corresponding Z_{90} values apply to clarithromycin, ibuprofen, iomeprol, iopamidol, metoprolol, sulfamethizole, trimethoprim and venlafaxine. Based on these findings, we may consider that Z_{90} can be used as an index to evaluate the efficient removal of pharmaceutical by ozonation.

4.3 Removal of natural fluorescence indicators

Fluorescence can be used as a tracer for the source fraction of DOC and its transformation during ozonation (Hudson, Naomi; Baker, Andy; Reynolds, 2007). Previous research has indicated that fluorescence can be used as a monitoring tool to determine indirectly ozone in recirculating aquaculture system water (Spiliotopoulou et al., 2017). The detailed introductions regarding fluorescence and the specific wavelengths used in this study are described in Papers IV and V.

Hence, the same ozonated samples from the pilot and laboratory experiments with different ozone doses were measured in six fluorescence wavelengths, consisting of protein-like and humic-like fluorophores (Figure 4.3).

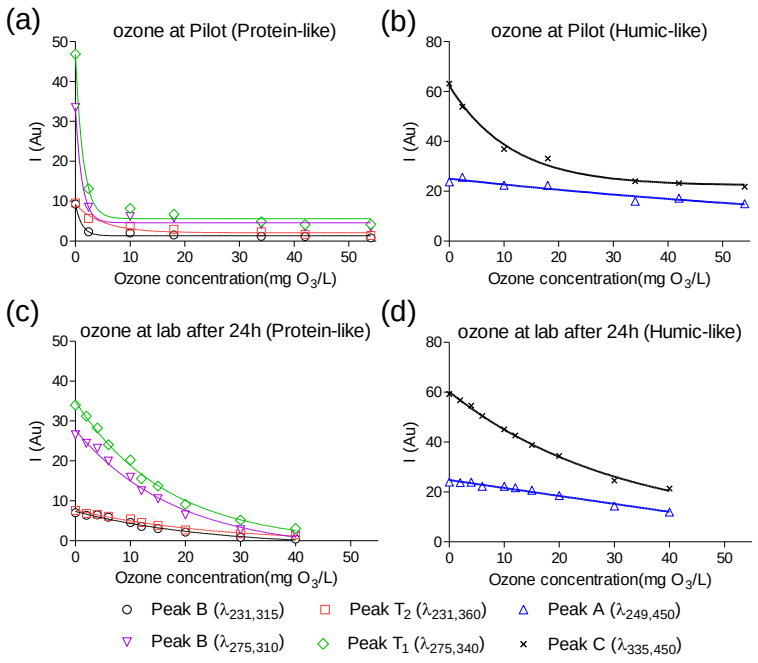

Figure 4.3. (a)-(d): comparison of remaining natural fluorescence of MBBRs effluent treated by ozone, using the pilot or laboratory method. (**Paper IV**)

Generally, if looking at the trend of fluorescence intensity in these two studies, the intensity of both the protein and the humic-like fluorophores decreased in line with an increase in ozone concentration. This decreasing fluorescence intensity can be explained by the depletion of or variation in aromatic structures and the increase of electron withdrawing groups such as COOH in aromatic compounds (Świetlik and Sikorska, 2004). In the pilot Herning study, the intensity of all protein-like fluorescent peaks decreased

significantly with ozone dosages around 2-10 mg O_3/L, whereas, for the laboratory experiments, the intensity of all protein-like fluorescence peaks decreased gradually and ended up at about 40 mg O_3/L. However, the intensity of humic-like fluorescent could not be removed in all instances, even at high ozone concentrations (40 or 50 mg O_3/L), because humic-like fluorescence stands for the least degradable organic matter in wastewater. Similar experimental findings regarding changes on the fluorescence intensity of protein-like and humic-like fluorophores were also observed in the Skejby experiments. Therefore, in order to predict and control water quality in online systems, less ozone is required when aiming at removing protein-like fluorescence, while high levels of ozone are needed when looking to remove humic-like fluorescence.

Additionally, in the Herning experiments, when investigating the development of fluorescence intensity over time in the wastewater treated in the laboratory through ozone and a polishing MBBR, the results were different compared with the standalone ozone process (Figure 4.4).

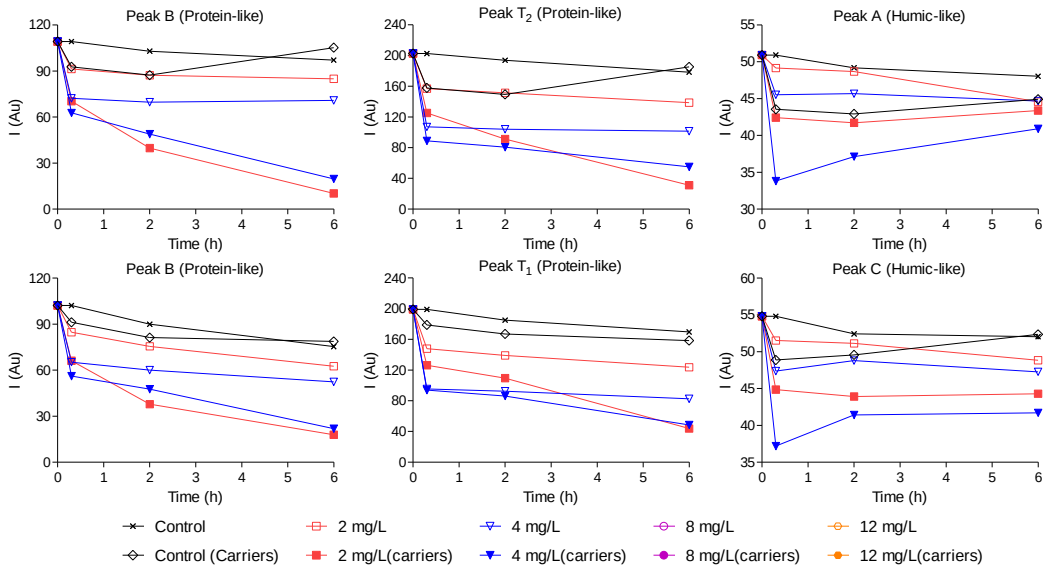

Figure 4.4. Development of natural fluorescence of MBBRs effluent treated by a single polishing MBBR, four doses of ozone in the laboratory, with or without subsequent MBBR polishing. (**Paper V**)

Normally, MBBRs effluent polishing with ozone and a subsequent MBBR can bring fluorescence intensity down to great extent compared to ozone treatment only. When increasing the contact time between ozonated wastewater and the subsequent MBBR, the relevant fluorescence intensity

gradually decreased. However, for humic-like fluorescent, the intensity of ozonated wastewater with a subsequent MBBR slowly increased until termination of the experiment, because during the MBBR polishing process, the attached biofilm started to detach and eventually dissolved in the wastewater, thereby contributing some fluorescence intensity. Overall, based on this laboratory test, ozone followed by a polishing MBBR is able to purify wastewater further and improve the quality of any discharge into receiving water.

4.4 Performance of micro-toxicity

Microtoxicity tests at 15 min of exposure time were carried out to investigate wastewater characteristics for the Herning staged MBBRs process and onsite continuous ozonation (Figure 4.5). Basically, the inhibition of toxicity was reduced on a tank by tank basis. The inhibitions of toxicity in ozonated wastewater first decreased and then increased again in both studies when ozone dosage increased. In this case, we can assume that highly toxic ozone by-products, compared to original substances, gradually generated when increasing ozone up to high concentrations. However, low concentrations of ozone were able to reduce half of the toxicity from MBBRs effluent.

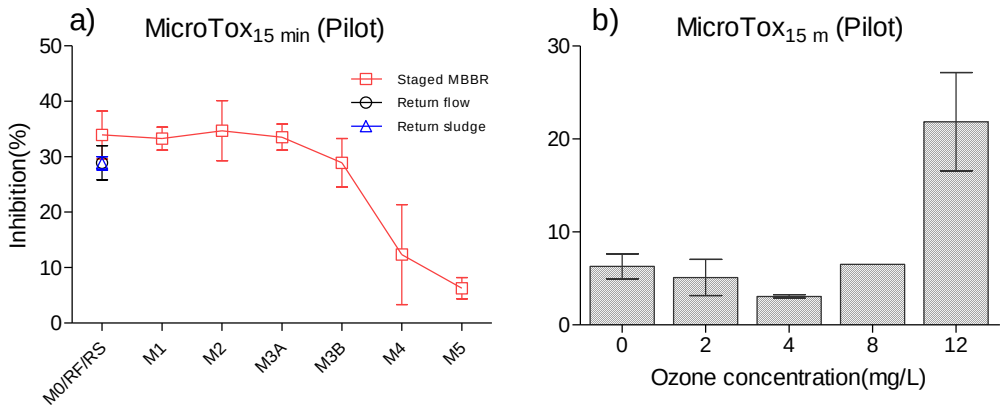

Figure 4.5. Toxicity measured with a MicroTox® test with 15 min exposure time in a pilot-scale MBBR treatment train (a) and onsite continuous pilot ozonation (b). M0: municipal influent fed into the pilot-scale MBBR treatment train. RF: return flow from M3B, marked with a black circle. RS: return sludge flow from a settling tank after M3B, marked with a blue triangle. M5: effluent from the MBBR treatment train, which was also the initial inlet for ozonation (O_3 =0 mg/L). (**Paper V**)

In the Herning study, a pilot polishing MBBR was applied in order to purify the ozonation effluent further. A comparison of toxicity inhibition between

ozonated wastewater and ozonated wastewater with a polishing MBBR is presented in Figure 4.6. In Figure 4.6a, the MBBR effluent was treated with different concentrations of ozone followed by a pilot polishing MBBR. Inhibitions decreased in line with increasing ozone concentrations, from 2 to 4 mg O_3/L, while they increased again when the applied ozone concentration reached 8 to 12 mg O_3/L, before gradually decreasing from 15 to 30 mg O_3/L. In the first stage, toxic DOC in ozonated effluent was reduced when increasing ozone concentration, while in the second stage, DOC was gradually reduced down close to zero. Simultaneously, ozone by-products were the main source of toxicity, and so the higher amount of ozone applied in the effluent, the higher the toxic ozone by-products produced, which led to an increase in inhibition. In the last stage, with ozone concentrations, inhibition decreased again, due to existing ozone by-products removed by a sufficient amount of ozone. For each ozone concentration applied, the subsequent polishing MBBR was able to bring down the remaining toxicity in ozonated effluent to almost zero, indicating that polishing MBBRs offer a very important and efficient way of enhancing the purification of ozonated effluent.

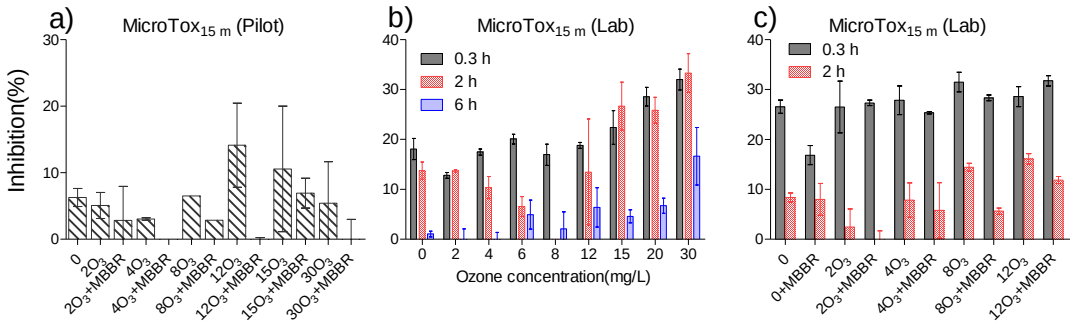

Figure 4.6. Comparison of MicroTox® with 15 min exposure time with different concentrations of ozone from the pilot and the laboratory experiments, followed by relevant pilot-scale or lab-scale MBBR polishing over time. (a) Staged MBBR effluent treated by onsite continuous pilot-scale ozone with various doses and a subsequent pilot MBBR polishing tank, (b) staged MBBR effluent treated by eight doses of ozone in the laboratory, (c) wastewater effluent treated by a single polishing MBBR, four doses of ozone in the laboratory, with or without subsequent MBBR polishing. (**Paper V**)

To understand further the features of microtoxicity development in ozonated wastewater, with or without polishing MBBR afterwards, the same MBBR effluent was ozonated with various doses in the laboratory, and the inhibition of individually ozonated samples decreased over time (Figure 4.6b). Over a 2-hour ozone reaction time, the inhibition of ozonated samples first decreased

and then increased when ozone concentration increased, which fitted to the first and second stages mentioned above. In Figure 4.6c, MBBR effluent was treated with different concentrations of ozone followed with a polishing MBBR in the laboratory. Over 2 hours' contact time, each ozonated sample treated with a polishing MBBR was less inhibited compared to the ozone-only samples, which again verified that the polishing MBBR is able to reduce further the toxicity of ozonated effluent.

5 Conclusions

The main findings of this thesis can be concluded as follow:

A pilot-scale staged MBBRs with both denitrification and nitrification processes, was proven as a feasible and effective solution to easing the effects of hospital wastewater on the environment. On the one hand, general parameters of wastewater, such as TOC, NH_4^+–N and NO_2^--N, were removed to a high extent, and thus this onsite MBBRs system was able to reduce treatment loading in conventional WWTPs, as the treated MBBRs effluents eventually discharge into the municipal sewer system. On the other hand, the higher k_{bio} of the majority of pharmaceuticals was observed for either denitrification or nitrification when compared to other similar researches on activated sludge and MBBRs. More than 50% of pharmaceuticals above LOQ in continuous flow experiments were removed. For diclofenac as a refractory compound, through intermittent feeding into the M3A/B reactors, its removal at 80% was two times higher compared to average removal in activated sludge or MBR.

Humic acid, simulating an externally complex carbon source, can affect the biodegradation rates of pharmaceutical in effluent treated by a laboratory-scale polishing MBBR as a tertiary treatment process. Twelve out of 24 investigated pharmaceuticals discovered as biodegradable compounds degraded faster with an increase in TOC derived from humic acid addition, thereby indicating that co-metabolism plays an important role, rather than competitive mechanisms, during pharmaceutical biodegradation. Similar conclusions have also been reached in previous research, where pharmaceuticals were oxidised by an enzyme or a co-factor produced during the metabolism of another growth substrate. The average stimulation of the first-order rate constant for biodegradable pharmaceuticals was 5% per mg DOC. 30 mgC/L by humic acid addition enhanced about three times the biodegradation performance of the laboratory-scale MBBRs.

By implementing the intermittent feeding of laboratory-scale MBBR reactors with raw settled wastewater and WWTP effluent for the first time, not only can MBBRs purify wastewater in relation to the high removal of ammonia, but degraders with the ability to biodegrade diclofenac present in wastewater are also promoted through an increase in biomass. Thus, the 2.1 h half-life of diclofenac, discovered in the intermittent feeding MBBRs system, was shorter than any wastewater bioreactor treatments to date, and its k_{bio} was more than ten times higher than in other similar studies. Moreover, for beta blocker

compounds such as those found in atenolol and metoprolol, their k_{bio} was also significantly higher compared to previous studies.

Ozonation was a feasible enforcement method for polishing the effluent of staged MBBRs and most pharmaceuticals remaining in MBBR effluent were removed when increasing ozone levels. Z_{90}, deriving from the normalisation of ozone doses with DOC, was used as a measuring index to compare the removal efficiency of pharmaceuticals in different ozonation experiments, and it can be considered to evaluate ozone performance for pharmaceutical removal. Fluorescence intensity, positively relating to BOD in wastewater, can also be considered as an index of water quality when using ozonation to purify wastewater. Based on experimental results, less ozone was needed when looking to reduce the fluorescence intensity of protein-like fluorophores; however, high amounts of ozone were required when reducing the fluorescence intensity of humic-like fluorophores. Pilot-scale staged MBBRs were capable of reducing toxicity inhibitions in wastewater, reactor by reactor, and subsequent ozone further reduced by half any toxicity remaining in MBBRs effluent. A polishing MBBR as a tertiary treatment solution, conjugated with ozone, was used to purify ozone effluent, and its toxicity caused by ozone by-products was entirely removed. Accordingly, ozonated wastewater treated with the polishing MBBR afterwards generally had the lowest intensity of protein-like fluorescence.

6 Future perspectives

The issue of a lack of sufficient biomass in effluents was addressed for the first time through the novel approach, which is intermittent feeding to laboratory-scale MBBRs with raw settled wastewater and WWTP effluent. Therefore, diclofenac a hardly degradable compound in effluent has achieved a half-time of degradation shorter than resulting from other biological reactors. Then, if the experimental methodology were scaled up from the laboratory to full scale, where the conditions and matrices of wastewater are much more complicated, it's interesting to know whether short half-life time of diclofenac can be still achieved or not if the novel feeding approach above could carry out in a full scale polishing MBBR.

In this study, an on-site pilot of staged MBBRs was carried out to treat raw hospital wastewater, and generally it was able to remove investigated pharmaceuticals to a high degree along with an overall reduction in wastewater toxicity. Implementing an on-site MBBR to treat wastewater, resulting in ease further loading process of WWTPs, can also be promote to treat other types of wastewater (i.e. wastewater in pharmaceutical factory) rather than only hospital wastewater in this study.

Natural fluorescence intensity in wastewater is character trait of BOD. The relationship between ozone dosage and natural fluorescence intensity was understood in this study by conducting off-line experiments in the laboratory, and thus fluorescence intensity could be recognised as a parameter/index of wastewater quality during ozonation. For technicians working in WWTPs, off-line experiments measuring fluorescence intensity for ozonated samples are feasible, but they are quite time-consuming. Hence, if an online sensor for detecting fluorescence intensity could be developed, wastewater quality during ozonation could be controlled based real-time data for fluorescence intensity. Furthermore, an optimised dose of ozone, achieving discharge standards for BOD, namely a higher ozone dose and less fluorescence intensity. Additionally, the relationship between fluorescence intensity and pharmaceutical concentration in wastewater is also interesting to interpret. If there were a coherent relationship, basic concentrations of pharmaceuticals will be able to indirectly calculate based on fluorescence intensity, and subsequent traditional analysis of pharmaceuticals could be omitted.

In this study, the MBBRs were generally run over a year or even more, and the effect of temperature on performance of MBBRs can't omit, especially

there is big temperature difference in summer and winter in Denmark. There were 5℃ differences in previous operation of MBBRs, which could affect performances of MBBRs on removal of pharmaceuticals, reduction of toxicity and fluorescence intensity. Therefore, in future, clear differences regarding the above performances in summer and winter need to be understood.

7 References

Antoniou, M.G., Hey, G., Rodríguez Vega, S., Spiliotopoulou, A., Fick, J., Tysklind, M., la Cour Jansen, J., Andersen, H.R., 2013. Required ozone doses for removing pharmaceuticals from wastewater effluents. Sci. Total Environ. 456–457, 42–49. doi:10.1016/j.scitotenv.2013.03.072

Asuero, A.G., Sayago, A., González, A.G., 2006. The Correlation Coefficient: An Overview. Crit. Rev. Anal. Chem. 36, 41–59. doi:10.1080/10408340500526766

Berger, K., Petersen, B., Buening-Pfaue, H., 2008. Persistence of drugs occurrring in liquid manure in the food chain. Arch. fuer Leb. (Germany, F.R.).

Blaney, L., 2014. Ozone Treatment of Antibiotics in Water, Water Reclamation and Sustainability. doi:10.1016/B978-0-12-411645-0.00012-2

Carvalho, R.N., Ceriani, L., Ippolito, A., 2015. Development of the first Watch List under the Environmental Quality Standards Directive water policy. doi:10.2788/101376

Cydzik-Kwiatkowska, A., Zielińska, M., 2016. Bacterial communities in full-scale wastewater treatment systems. World J. Microbiol. Biotechnol. 32, 1–8. doi:10.1007/s11274-016-2012-9

Dantas, R.F., Canterino, M., Marotta, R., Sans, C., Esplugas, S., Andreozzi, R., 2007. Bezafibrate removal by means of ozonation: Primary intermediates, kinetics, and toxicity assessment. Water Res. 41, 2525–2532. doi:10.1016/j.watres.2007.03.011

Escolà Casas, M., Chhetri, R.K., Ooi, G., Hansen, K.M.S., Litty, K., Christensson, M., Kragelund, C., Andersen, H.R., Bester, K., 2015a. Biodegradation of pharmaceuticals in hospital wastewater by staged Moving Bed Biofilm Reactors (MBBR). Water Res. 83, 293–302. doi:10.1016/j.watres.2015.06.042

Escolà Casas, M., Chhetri, R.K., Ooi, G., Hansen, K.M.S., Litty, K., Christensson, M., Kragelund, C., Andersen, H.R., Bester, K., 2015b. Biodegradation of pharmaceuticals in hospital wastewater by a hybrid biofilm and activated sludge system (Hybas). Sci. Total Environ. 530–531, 383–392. doi:10.1016/j.scitotenv.2015.05.099

Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A., la Cour Jansen, J., 2012. Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. Water Res. 46, 1167–1175. doi:10.1016/j.watres.2011.12.003

Falås, P., Longrée, P., la Cour Jansen, J., Siegrist, H., Hollender, J., Joss, A., 2013. Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process. Water Res. 47, 4498–4506. doi:10.1016/j.watres.2013.05.010

Hansen, K.M.S., Spiliotopoulou, A., Chhetri, R.K., Escolà Casas, M., Bester, K., Andersen, H.R., 2016. Ozonation for source treatment of pharmaceuticals in hospital wastewater – Ozone lifetime and required ozone dose. Chem. Eng. J. 290, 507–514. doi:10.1016/j.cej.2016.01.027

Herrmann, M., Olsson, O., Fiehn, R., Herrel, M., Kümmerer, K., 2015. The significance of different health institutions and their respective contributions of active pharmaceutical ingredients to wastewater. Environ. Int. 85, 61–76. doi:10.1016/j.envint.2015.07.020

Hollender, J., Zimmermann, S.G., Koepke, S., Krauss, M., Mcardell, C.S., Ort, C., Singer,

H., Von Gunten, U., Siegrist, H., 2009. Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. Environ. Sci. Technol. 43, 7862–7869. doi:10.1021/es9014629

Hudson, Naomi; Baker, Andy; Reynolds, D., 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. River Res. Appl. 23, 631–649. doi:10.1002/rra.1005

Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell-Marquet, C., Browning, S., 2008. Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England. Sci. Total Environ. 391, 149–158. doi:10.1016/j.scitotenv.2007.10.054

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H., 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: Consequences for plant optimization. Environ. Sci. Technol. 38, 3047–3055. doi:10.1021/es0351488

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40, 1686–1696. doi:10.1016/j.watres.2006.02.014

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S., 2012. Hospital wastewater treatment by membrane bioreactor: Performance and efficiency for organic micropollutant elimination. Environ. Sci. Technol. 46, 1536–1545. doi:10.1021/es203495d

Ødegaard, H., 2006. Innovations in wastewater treatment: The moving bed biofilm process. Water Sci. Technol. 53, 17–33. doi:10.2166/wst.2006.284

Overturf, M.D., Anderson, J.C., Pandelides, Z., Beyger, L., Holdway, D. a., 2015. Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction. Crit. Rev. Toxicol. 45, 469–491. doi:10.3109/10408444.2015.1038499

Plósz, B.G., Langford, K.H., Thomas, K. V., 2012. An activated sludge modeling framework for xenobiotic trace chemicals (ASM-X): Assessment of diclofenac and carbamazepine. Biotechnol. Bioeng. 109, 2757–2769. doi:10.1002/bit.24553

Spiliotopoulou, A., Martin, R., Pedersen, L., Andersen, H.R., 2017. Use of fl uorescence spectroscopy to control ozone dosage in recirculating aquaculture systems. Water Res. 111, 357–365. doi:10.1016/j.watres.2016.12.036

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44, 3214–3224. doi:10.1016/j.watres.2010.02.040

Świetlik, J., Sikorska, E., 2004. Application of fluorescence spectroscopy in the studies of natural organic matter fractions reactivity with chlorine dioxide and ozone. Water Res. 38, 3791–3799. doi:10.1016/j.watres.2004.06.010

Ternes, T. a., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., Teiser, B., 2003. Ozonation: A tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37, 1976–1982. doi:10.1016/S0043-1354(02)00570-5

Tobajas, M., Monsalvo, V.M., Mohedano, A.F., Rodriguez, J.J., 2012. Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by Comamonas testosteroni. J. Environ. Manage. 95, S116–S121. doi:10.1016/j.jenvman.2010.09.030

Tran, N.H., Urase, T., Ngo, H.H., Hu, J., Ong, S.L., 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Bioresour. Technol. 146, 721–731. doi:10.1016/j.biortech.2013.07.083

Verlicchi, P., Al Aukidy, M., Galletti, a., Zambello, E., Zanni, G., Masotti, L., 2012a. A project of reuse of reclaimed wastewater in the Po Valley, Italy: Polishing sequence and cost benefit analysis. J. Hydrol. 432–433, 127–136. doi:10.1016/j.jhydrol.2012.02.024

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012b. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. Sci. Total Environ. 429, 123–155. doi:10.1016/j.scitotenv.2012.04.028

Vieno, N., Sillanpää, M., 2014. Fate of diclofenac in municipal wastewater treatment plant - A review. Environ. Int. 69, 28–39. doi:10.1016/j.envint.2014.03.021

8 Papers

I Ooi, G.T.H., **Tang, K.,** Chhetri, R.K., Kaarsholm, K.M.S., Sundmark, K., Kragelund, C., Litty, K., Christensen, A., Lindholst, S., Sund, C., Christensson, M., Bester, K., Andersen, H.R., 2017. Biological treatment of hospital wastewater in a pilot-scale staged Moving Bed Biofilm Reactors (MBBRs) utilizing both nitrifying and denitrifying processes. *Manuscript to be submitted*.

II **Tang, K.**, Escola Casas, M., Ooi, G.T.H., Kaarsholm, K.M.S., Bester, K., Andersen, H.R., 2017. Influence of humic acid addition on the degradation of pharmaceuticals by biofilms in effluent wastewater. *International Journal of Hygiene and Environmental Health*, **220**, 604-610.

III **Tang, K.**, Ooi, G.T.H., Litty, K., Sundmark, K., Kaarsholm, K.M.S., Sund, C., Kragelund, C., Christensson, M., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals in conventionally treated wastewater by a polishing moving bed biofilm reactor (MBBR) with intermittent feeding. *Bioresource Technology*, **236**, 77-86.

IV **Tang, K.**, Spiliotopoulou, A., Chhetri, R.K., Ooi, G.T.H., Kaarsholm, K.M.S., Sundmark, K., Florian, B., Kragelund, C., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals, toxicity and natural fluorescence by ozonation of biological treated hospital wastewater with further polishing by suspended biofilm. *Manuscript to be submitted*.

V **Tang, K.**, Ooi, G.T.H., Chhetri, R.K., Spiliotopoulou, A., Kaarsholm, K.M.S., Sundmark, K., Florian, B., Kragelund, C., Bester, K., Andersen, H.R., 2017. Removal of pharmaceuticals, toxicity and natural fluorescence by ozonation in biological pre-treated municipal wastewater in comparison to subsequent polishing biofilm reactors. *Manuscript to be submitted*.